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MASTER'S THESIS

NASA Jet Propulsion Laboratory Hydrothermal Vent Bio-sampler

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Abstract

This MSc diploma thesis work in Space Engineering has been performed at the NASA Jet Propulsion Laboratory, where one of the projects included the construction and development of a hydrothermal vent bio-sampler.

On the bottom of the oceans with volcanic activity, hydrothermal vents can be found which spew out mineral-rich superheated water from the porous seafloor crust. Some of these vents are situated several thousand meters below the surface, where sunlight never reaches. Yet life thrives there on the minerals and chemical compounds, which the vent water brings up with it. This chemosynthetic microbial community forms the basis of some of the most interesting ecosystems on our planet, and could possibly also be found on other water-rich planets and moons in the solar system. Perhaps hydrothermal vents exist under the icy surface of the moon Europa with a biota thriving independently of the solar energy.

The Hydrothermal Vent Bio-sampler (HVB) will be a system to collect pristine samples of the water emanating from subsurface hydrothermal vents. An array of temperature and flow sensors will monitor the sampling conditions, which will allow for the samples to be collected from defined locations within the vent plume. From this the diversity and distribution of the chemosynthetic communities, which might live there, can be accurately described. The samples will have to be taken without any contamination from the surrounding water, thus the pristine requirement. Monitoring the flow will assure that enough water has been sampled to account for the low biomass of these environments. The system will use a series of filters, down to 0.2 μm in pore size, and the samples can be directly collected from the system for both culture- and molecular-based biological analyses.

The HVB now in its development and testing phase, is designed to operate under the extreme conditions at the bottom of the oceans by the hydrothermal vents. This means that the system needs to handle temperatures of up to 400 °C and pressures corresponding to the depth of about 7000 km below the ocean surface.

After testing at a hydrothermal vent system in the Eyjafjördur fjord off the coast of Iceland the previous year, modifications and improvements were made to the HVB. This system has, amongst others, now successfully passed the pressure test at Scripps Institution of Oceanography and an ocean test off the coast of Los Angeles. The system was also returned to Eyjafjördur on Iceland earlier this spring for a new test and to collect samples. These samples will now be analyzed to look for any possible biological signatures in them.

To my parents Arne and Britt-Marie

Our time will be remembered, because this was when we first set sail for other worlds.
—Carl Sagan

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Thank you very much!

Acronyms

A	Ampere
B	Byte
b	bit
BCD	Binary Coded Decimal
DSN	Deep Space Network
EEPROM	Electrically Erasable Programmable Read Only Memory
EME	Electronically Monitored Ecosystems
GND	Ground in electrical circuits
GUI	Graphical User Interface
HVB	Hydrothermal Vent Bio-sampler
I^2C	Inter-Integrated Circuit
IC	Integrated Circuit
I/O	Input/Output
JPL	Jet Propulsion Laboratory
LCD	Liquid Crystal Display
LED	Light Emitting Diode
MBARI	Monterey Bay Aquarium Research Institute
MER	Mars Exploration Rover
MGS	Mars Global Surveyor
MRO	Mars Reconnaissance Orbiter
NASA	National Aeronautics and Space Administration
OAR	Ocean Atmosphere Research
PBASIC	Parallax Beginner's All-purpose Symbolic Instruction Code
PCB	Printed Circuit Board
PCR	Polymerase Chain Reaction
PSI	Pounds per Square Inch
PVC	Polyvinyl chloride
PWM	Pulse Width Modulation
RAM	Random Access Memory
ROM	Read Only Memory
RTC	Real Time Clock
SIO	Scripps Institution of Oceanography
SPI	Serial Peripheral Interface
SSR	Solid State Relay
V	Volt
VDC	Volt in Direct Current
VI	Virtual Instrument
WHOI	Woods Hole Oceanographic Institute

Chapter 1

Introduction

This thesis work describes the Hydrothermal Vent Bio-sampler (HVB) instrument, currently under construction and development at NASA Jet Propulsion Laboratory (JPL) in Pasadena, California, USA. This report will provide an introduction of the HVB system, a background to the project and the work that has been done the past six months.

1.1 The hydrothermal vent bio-sampler

Scientists say we know more about what exists outside of our Earth's atmosphere than what can be found here under our own sea surface. Hydrothermal vents can be found in geological active regions on the bottom of the oceans. These are geysers that spew out mineral-rich superheated water from below the ocean floor. At these depths, where sunlight never reaches, an amazing biota can be found at the hydrothermal vents. Micro organisms live and thrive off the minerals provided by the vent water, an oasis in an otherwise barren landscape. With the discovery of this alien world on our own Earth, it is not hard to stretch one's imagination that there could also be places like this elsewhere in the solar system. Hydrothermal vents could, for example, exist near still active volcanoes on Mars or in an ocean under the frozen crust of Jupiter's moon Europa, where NASA plans to send a probing spacecraft.

Due to the hostile and hard to reach environment, the exploration of the submarine hydrothermal vents has been possible only through the usage of deep submarine technology. Using robots to explore, photograph and take samples has greatly increased our knowledge and understanding of the activities far below the surface of the oceans.

The HVB project is a part of NASA's Search for Origins of Life program, which focuses on developing high-level discovery missions based on results and concepts of projects that prove their reliability and efficiency here on Earth.

The HVB is to probe the plume of up to 400 °C deep-sea hydrothermal vents, to perform in-situ measurements and collect pristine samples of the vent water emanating for laboratory bio-analysis. The HVB system will help enhance our knowledge of life in extreme environments and to develop a probing and pristine sampling routine for terrestrial and extraterrestrial exploration. It will contribute to our knowledge in the field of astrobiology and the search for

extraterrestrial life in the universe.

The HVB system is built with the extreme hydrothermal vent environment in mind, needing to withstand extreme temperatures and pressures. This project would require deep-sea technology and specialized components, but due to budget restrictions off-the-shelf components are used to build up the HVB. This project is currently in its development phase, constantly evolving and reconfigured after tests and upgrades.

Analyses will be performed on the recovered samples from the vents with validated bio-assessment methods and protocols. A discovery of microbes in the superheated vent water plumes will set a new limit to the boundary of life as we know it and extend the possibilities for finding life elsewhere in the universe.

1.2 The Jet Propulsion Laboratory

Far away, near the edge of our solar system two spacecraft are making their way towards the heliopause. Launched almost 30 years ago, Voyager 1 and 2 keep sending their data home to Earth and the place where they were once born; NASA Jet Propulsion Laboratory (JPL) in Pasadena, CA, USA.

Figure 1.1: The entrance to NASA JPL.

The history of JPL goes back to 1926 when California Institute of Technology (Caltech) created an aeronautics graduate school after receiving a $300,000 grant. From this, the Guggenheim Aeronautical Laboratory of the California Institute of Technology (GALCIT) was created, and a small group of graduate students started testing rocket propulsion. At first the group had to fund their project themselves, but with the onset of World War II, governmental funding started to flow in.

At first rockets and missiles were developed for military purposes, but with the launch of Sputnik in 1957, the control of the facilities were handed over to the newly formed National Aeronautics and Space Administration (NASA). The research now switched from rocket propulsion to the instruments to be carried into space and to alien worlds by them.

One of JPL's key objectives is the robotic exploration of space and to make robotic vehicles for the exploration of the universe, instead of by humans. This has lately been demonstrated by the very successful rover and orbiter missions to Mars, the Mars Exploration Rovers (MER), Mars Global Surveyor (MGS) and Mars Odyssey.

Now NASA's spacecraft and rovers are all being developed and built here at JPL. Mariner, Voyager and Galileo spacecraft, as well as the MER rovers are but a few of those that came out from these facilities. The latest addition to the family is now the Mars Reconnaissance Orbiter (MRO), which just made Mars gravity capture in March this year. After their launch these spacecraft are controlled with the help of the Deep Space Network (DSN), which is also managed from JPL.

The NASA JPL facilities are built on the San Andreas Fault, at the foot of the San Gabriel Mountains, Figure 1.2, and can be seen when driving down the 210 Freeway west out of Pasadena. Well inside the security gates, JPL has a relaxed atmosphere. The buildings lie along streets with names such as Surveyor and Explorer Road. Deer roam freely on the greeneries, sharing the campus-like area with the engineers and scientists working here. Inside the facilities spacecraft are being managed, and new exploration vehicles and technologies are developed and built.

Figure 1.2: NASA JPL facilities.

Chapter 2

Hydrothermal vents

Hydrothermal vents can be found in areas with volcanic activity on Earth, and most likely on several other planetary bodies in the solar system. Hydrothermal vents are reservoirs of water heated by magma, molten rock, underneath the surface and are fairly common here on Earth, because our planet is very geologically active and has a lot of water. How common this phenomenon is on other planets and moons is not yet known, but indications of water or other liquids and geological activities have been observed on the Galilean moons of Jupiter and the Saturnian moons Titan and Enceladus.

The red star-like object seen shining on our nigh skies, the planet Mars, could also be a host to these hydrothermal vents. Old volcanoes and water ice have been found there, and if some of these volcanoes are still geologically active, liquid water could exist beneath the surface in a hydrothermal vent-like environment.

What is so exciting and interesting about these hydrothermal vents is that these areas are thriving with life, even thousands of meters below the surface of the oceans. Life around these vents exists nearly independent of their surroundings and the sun. It is highly possible that life once upon a time started at a place such as this, by a nourishing hydrothermal vent protected from the harsh and dangerous environment above the surface [3]. Perhaps this is also where life exists elsewhere in the universe, just awaiting our discovery.

2.1 Geology

Hydrothermal vents are a result of water reservoirs being heated by geological activity such as volcanoes and hot spots, areas where the underlying magma is relatively close to the Earth's surface. This interaction results in hydrothermal activities on the surface, such as fumaroles, hot springs and geysers. Perhaps the most famous of all the hydrothermal vents is the Old Faithful geyser in Yellowstone Park, USA, Figure 2.1.

These geologically active regions can most commonly be found along the boundaries of the lithospheric plates, Figure 2.2. In the theory of plate tectonics the outermost part of our Earth consists of the solidified lithospheric layer, which, in turn, floats on the underlying asthenospheric layer. The lithospheric layer is split up into ten larger and several smaller plates, which move relative to

Figure 2.1: Old Faithful in Yellowstone Park in USA, Wikipedia.com.

each other. It is in the interaction of these plates, where new crust is constantly forming and old crust is consumed through subduction, where the underlying magma can more easily ascend to create geologically active regions on the surface. Since most of Earth is covered with water, hydrothermal vents exist also under the ocean surface, creating a unique environment.

Hot and strongly saline water, up to 60 °C, were first discovered in 1949 through a survey in the Red Sea. This water was found to be emanating from a rift on the seafloor, with surrounding mud containing higher concentrations of metals. In 1977, along the East Pacific Rise off the coast of South America, near the Galapagos Islands, the so called "black smokers" were first discovered by scientists using the deep-sea exploring submersible Alvin [19], Figure 2.3. Black smokers are the warmest and most active of the hydrothermal vents, Figure 2.4. Since then a number of these vents have been discovered and studied in both the Atlantic and the Pacific Ocean. They can be found at depths ranging from a couple of meters to several kilometers, averaging at about 2500 meters along the mid-ocean ridge system.

The mid-ocean ridge is formed where the lithospheric plates divert and magma is forced up by the convection currents underneath, literally lifting up the ocean floor. These ridges are all connected and create the longest continuous chain of volcanoes on Earth, some 50,000 km, and is thus Earth's longest mountain range. The diverging and creation of new ocean floor is a slow process with a typical spreading rate of around 60 mm a year, creating a total of 20 cubic kilometers of new ocean crust per year [11, 24].

The geological phenomenon subsurface hydrothermal vents is illustrated by

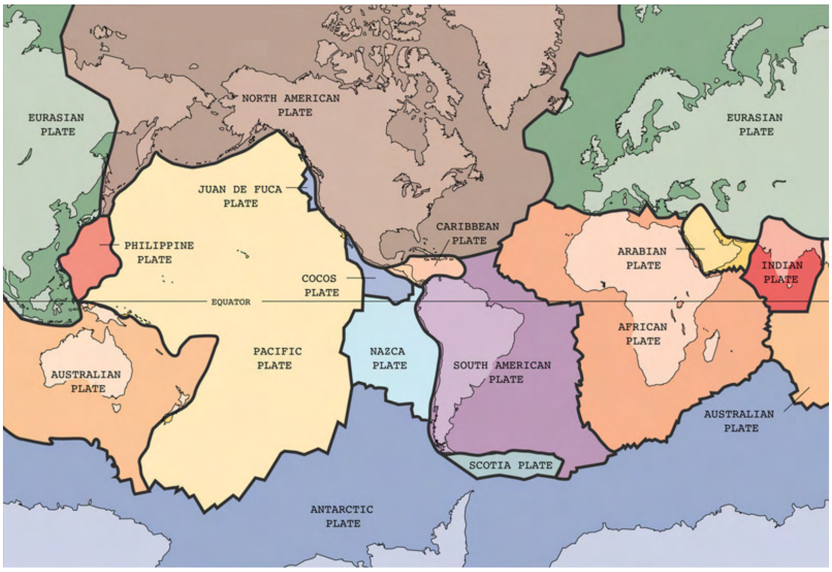

Figure 2.2: The lithospheric plates, Wikipedia.com.

Figure 2.5 [18]. The ocean floor is made up of porous volcanic rock. Due to the tectonic movements, cracks are created in the ten kilometer thick crust. Cold seawater, at approximately 2 °C, flows down through these cracks and becomes superheated by the thousand degrees hot magma underneath the thin crust, to a temperature of up to 400 °C. Superheating is when a liquid is headed to a temperature above its standard boiling point without starting to boil. At sea level, with one atmospheric pressure, the boiling point of water is 100 °C. The pressure increases by one atmosphere for every ten meters of water under the surface. At a depth of 3000 meters the pressure is about 300 atmospheres and the boiling point has increased to just over 400 °C.

With the higher temperature the water in the porous crust reacts with its surroundings. At about 60 °C the rock is oxidized by the water, meaning that oxygen is removed from the water. Other reactions also take place and elements, such as potassium are removed from the seawater. At about 300 meters below the seafloor the temperature reaches 150 °C. Here the water becomes more acidic and elements such as calcium sulfate and magnesium are removed. When the water reaches even deeper down into the crust and to higher temperatures, sodium, calcium and potassium are dissolved in the water from the surrounding rock. If the water keeps going even further down it reaches temperatures of 350–400 °C, and copper, zinc, iron and sulfur are dissolved in the acidic water from the rock. There are several reactions involved in the progress for the water through the crust, and the whole process is not yet fully understood. Eventually the water starts to rise, since warmer water is more buoyant than colder, and finally emerges up out of the crust through the vents.

The heated water that emerges from the bottom is mixed with the cold ambient water in the ocean. Metals that were brought up with the superheated water from below the seafloor now react with the sulfur in the ambient water, producing tiny particles of metal sulfides. These metals sink back to the bot-

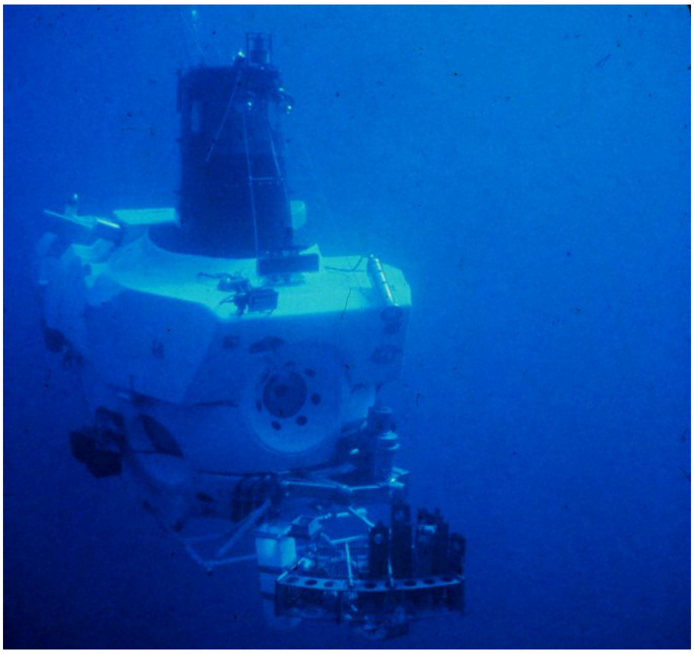

Figure 2.3: Deep-sea explorer Alvin, NOAA.

tom and form deposits on the ocean floor. These deposits are enriched mainly with copper, iron and zinc, but also with other metals such as barium, lead, gold and silver. Ancient sea floors, now exposed above the ocean surface, have mineral deposits such as this. These deposits contain also ancient fossilized animals, which once were totally engulfed and are now preserved in these mineral deposits.

The particles created build up chimney-like structures around the vents. These chimneys can grow very fast, some up to 30 cm per day. These structures are fragile and usually reach only some tens of meters before collapsing under their own weight. After the collapse the process starts all over again, and soon another chimney stands tall at the site. One of the most famous chimney structures was called Godzilla. This was a chimney off the coast of Oregon in the Pacific Ocean, which reached a height equal to a 15 story building before collapsing.

The warmest and most active hydrothermal vents are known also as black smokers. The name comes from the metal sulfide particles, which create a black smoke-like plume from the vent. The water emanating from these vents can reach temperatures of up to 400 °C.

In some cases the superheated water mixes with the colder and oxygen richer seawater under the surface, before emanating through the vents. The metal sulfides are then also created earlier and do not reach the surface. Instead other compounds are brought up through the chimney, such as silica and anhydrite, and these minerals create a white smoke. Thus these vents are called white smokers. The water emanating from these vents are colder, with temperatures of 250–300 °C, and the flow is slower than for the black smokers. The chimneys are also smaller than those created by the black smokers.

Figure 2.4: Black-smoker hydrothermal vent, WHOI.

Some hydrothermal fluids do not pass through the chimneys with the fast hot flow but instead seeps out at other places in the ocean floor. The mixing with seawater takes place within the crust, and the flow is even slower, so much of the minerals are left inside the crust. These outflows can be seen as a transparent shimmering of the much warmer vent water plume in the ambient cold seawater. However, the small amount of sulfides which is brought out, as with the other vents described above, are what provides the nourishment for the microorganisms living around these sites.

2.2 Biology

With the discovery of the hydrothermal vents deep below the sea surface, scientists made another remarkable discovery. Despite the toxicity of the metals and the extreme temperatures of the vent water, life was thriving there, far out of the reach of the sunlight, in a total darkness and in an otherwise barren landscape. The scientists figured that the nutrients transported from the surface down to these areas were not enough to feed all this life. Instead scientists found that life around the vents were living of the minerals and chemical compounds emanating with the hydrothermal vent waters.

2.2.1 Microbes

Microbes were found to live everywhere; on rocks, inside the chimneys and even in symbiosis inside other larger animals. The microbes include bacteria and archaea, the latter being one of the most ancient forms of life. The archaea and bacteria are prokaryotes, simple cells lacking a nucleus. Their genetics is very different from each other and from the eukaryotic life, such as mammals and plants, and thus have their own domains on the tree of life. The discovery raises the question if life could have started at one of these hydrothermal vents

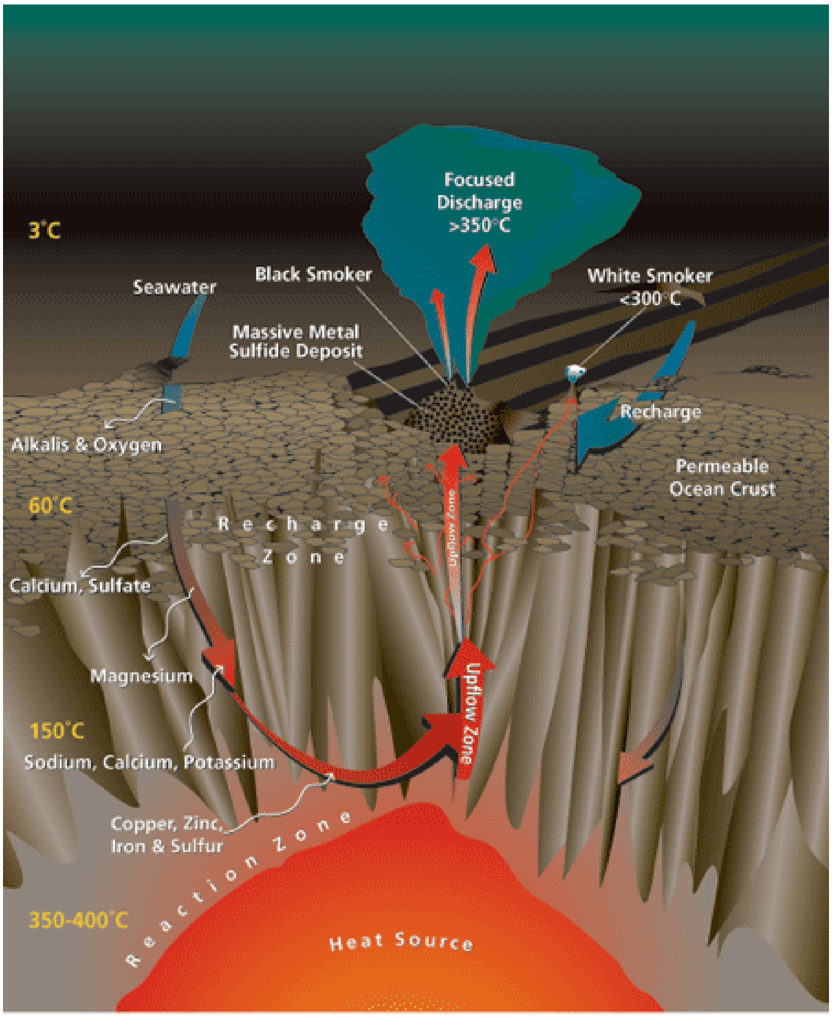

Figure 2.5: Hydrothermal vents cycle, WHOI.

some four billion years ago here on Earth, deep under the surface of an ocean, protected from the deadly solar radiation and meteorite strikes [3].

The microbes live at the vents by using chemosynthesis, which is analogous to photosynthesis used by plants, shown in Equation 2.1. The photosynthesizing plants use the energy from the sun, water and carbon dioxide in order to produce sugar and oxygen. In a similar way the chemo-synthesizing microbes break the bonds of different chemicals to release the energy and transform this to the fuel of life, sugar:

$$CO_2 + 2H_2O + energy\ from\ light \rightarrow CH_2O + O_2 + H_2O \qquad (2.1)$$

Different microbes use different chemicals and minerals to produce the organic compounds. There are many sulfide oxidizing microbes that use the chemical sulfides from the vents for their metabolism. By breaking the chemical bonds of the hydrogen sulfide and combining it with oxygen and carbon dioxide from

the seawater they produce carbohydrate sulfur and water. The carbohydrate, CH_2O in Equation 2.2, is used as fuel.

$$CO_2 + O_2 + 4H_2S \rightarrow CH_2O + 4S + 3H_2O \tag{2.2}$$

Some vents have been nicknamed "snow-blowers" because of all the particle-like microbes emanating from the vents, resembling a snow blower. The microbes form the basis of the food chain for the animals living around the vents. Other larger animals use these microbes inside their bodies in a symbiotic relationship. For example, microbes reside in the gut of the tubeworms and in the gills of the clams. Inside these larger animals the microbes get a safe haven in exchange for the nutrients that they produce and provide with for the host. This relationship has even gone so far that the tubeworms do not have mouths or stomachs, but are fully dependent on the microbes to provide the nutrients for them. The other animals that do not depend on any symbiotic relationship with the microbes graze the areas around the vents for bacterial scum or filter the bacteria out from the vent water. Above these animals in the food chain are also predators and scavengers, feeding on the other larger animals, not directly dependent on the hydrothermal vents.

The microbes living in these extreme temperatures, so-called extremophiles, are of great interest, even to the industry. Their enzymes can be useful in high temperature applications, such as food processing, drugs, paper manufacturing and dislodging oil inside wells.

2.2.2 The other animals

Before the discovery of the hydrothermal vents and their biota, the Sahara desert was believed to host the most heat-resistant animals on Earth. The champion here was the Sahara desert ant (*Cataglyphis bicolor*), able to withstand temperatures of 55 °C. But the animals living around the hydrothermal vent deep in the oceans cannot only survive the extreme pressure, but also the higher temperatures found here. The most extreme of these animals found thus far is the Pompeii Worm (*Alvinella pompejana*) seen in Figure 2.6. A temperature of 80 °C was recorded on the rear end of one of these worms sitting at a hydrothermal vent. The head of the worm, sticking out of its tube, was, however, situated in a much cooler area with a temperature of 22 °C.

The tubeworms, Figure 2.7, living at the hydrothermal vents can grow up to 2 meters long. They live their whole life inside their tubes, made of a hard material called chitin. This tube is used as protection against toxic vent chemicals and predators. As already stated, the tube worms do not have a mouth or a stomach, and therefore they cannot eat by themselves. Instead they harbor microbes that produce the nourishment for them, in exchange for the protection in their tubes. The tubeworms provide these microbes with the hydrogen sulfide and oxygen, which they absorb with their gill-like plumes, and the microbes use these chemicals to produce sugar, such as in Equation 2.2. The tubeworms have been found to be one of the first animals to populate a new vent site, but it is still unknown how they arrive to these new sites, or even how they reproduce.

Mussels live at hydrothermal vents, usually colonizing a crack, and are the first shellfish at a new hydrothermal vent. They can move by shooting out a thread that sticks to a surface, which they then reel themselves in with. Mussels

Figure 2.6: The Pompeii Worm, Wikipedia.com.

also live in symbiosis with the microorganisms that produce sugar for them, but they can also filter the surrounding water for nutrients to survive for shorter periods of time.

Clams arrive to the hydrothermal vents after the mussels. They live much like the mussels, off the sugar provided from the symbiosis with the microbes. Instead of using threads to move around, clams are equipped with a muscular foot. This appendix can also be used to fit into and anchor them to cracks at the vents.

There are shrimps, sometimes a shoal of thousands of animals, which gather around the vent and feed from the microbes. Crabs also like hydrothermal vents. They are scavengers and predators and eat off the other lifeforms that can be found around the vents.

One of the top predators at the hydrothermal vents are the octopi. These are a common animal in seas around the world but there are some species that are unique to the hydrothermal vent sites. Another top predator is the zoarcid fish, Figure 2.8. This is a 60 cm long, slow and sluggish fish that feed of the other lifeforms around the vents.

The Dandelions are a jellyfish-like colony of several individuals grouped together into a spherical formation. These animals are scavengers and use their tentacles to anchor themselves to the ground. These animals are the last to colonize a vent. If many of them are found it is most likely that the vent has gone out and the life around it is dying.

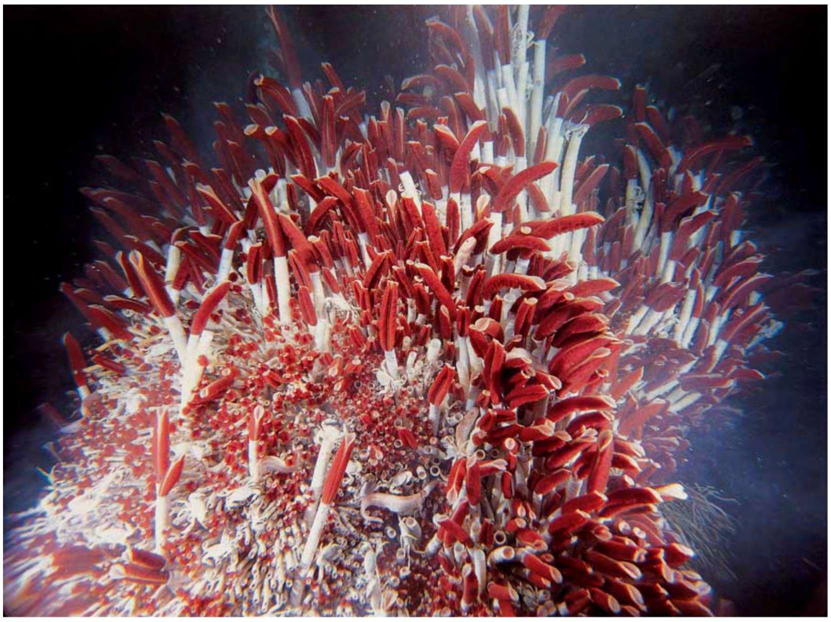

Figure 2.7: Colony of tube worms at a diffuse flow vent, Barakay Laboratory.

Figure 2.8: The zoardic fish, WHOI.

Chapter 3

Vents beyond Earth

As has been seen above, life can exist at isolated places deep down at the bottom of the oceans, in an otherwise so barren landscape. It is not hard to stretch ones mind and imagine that there could be other worlds with hydrothermal vents that can harbor such a thriving biota.

Life as we know it requires liquid water. Frozen water-ice has been found to exist on several other planetary bodies in the solar system. If these planets and moons are also geologically active, providing a source of heat, then there is also likely that liquid water can exist around these sites. Thus, a geological process similar to the hydrothermal vents here on Earth could provide an environment where life could flourish also elsewhere in the solar system.

3.1 Europa

One of the best candidates for extraterrestrial life in the solar system is the moon Europa. This is the second of the four Galilean moons of Jupiter, as seen from the planet. The four largest moons of Jupiter (Io, Europa, Ganymedes and Callisto) are called the Galilean moons after their discoverer Galileo Galilei.

The innermost moon Io may be the most geologically active body in the solar system. Io has a high geological activity through a process called tidal heating and orbital resonance [3]. Tidal heating is frictional heating of the interior of a body due to the gravitational pull of another larger body, and possibly also of other neighboring bodies. In this case the main body responsible for this effect is Jupiter. Orbital resonance is when the period of a body is related to that of another one by a simple integer fraction. This is true for the three inner Galilean moons, which line up with each other every seven days.

The forces acting on Io result in a surface freckled with volcanoes and temperatures inside the body high enough to melt rock. The environment created on Io by these forces is thought to be too harsh for any life. However, further out, the other three Galilean moons are also affected by these forces, but to a lesser extent. These moons all have ice-covered surfaces, but it is not known what the conditions are underneath. There might be just enough geological activity on these moons to create a liquid ocean underneath the ice-covered surface.

3.1.1 Oceans on Europa

Europa is a cold place, slightly smaller than Earth's moon, and is covered with a crust of water ice, Figure 3.1. Measurements from the Galileo spacecraft indicate that the moon has undergone differentiation in its early history. This means that heavier elements have collected in the center, such as rocks and metals, and that the lighter elements, such as water, are collected in an outer shell, Figure 3.2. This outer shell, which consists mostly of water-ice, is thought to be 80–170 km thick. The measurements of Galileo did not, however, reveal the state of the water underneath the surface, but there are indications that it could be liquid or something in between, like slushy partially melted ice [20]. It is known that the surface is frozen, since the moon has a surface temperature of -150 °C. The surface is very smooth, not changing in height by more than a few kilometers. It is estimated that this frozen topmost crust extends down 5–25 kilometers, but it could also be thinner.

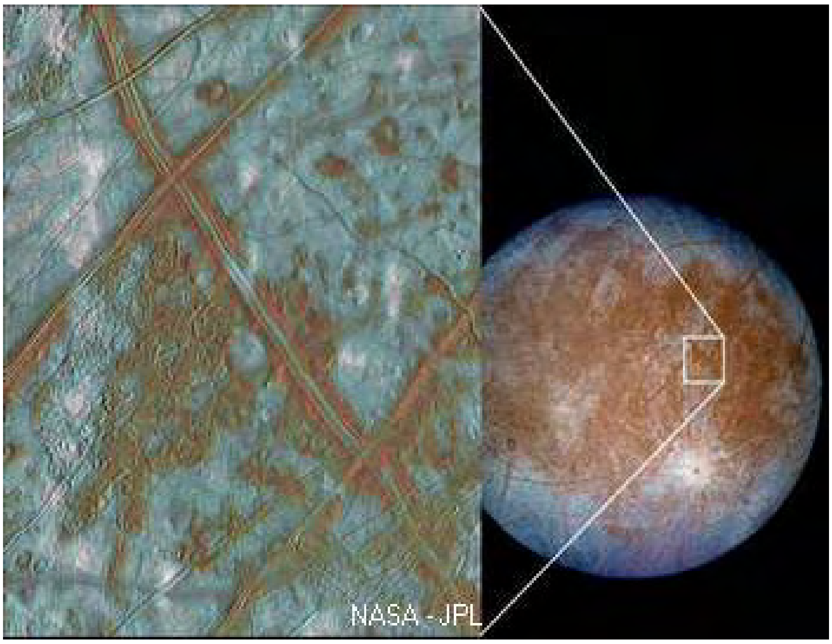

Figure 3.1: Europa and its icy surface, NASA JPL.

Europa's surface lacks the number of impact craters one would expect from a body without a thicker atmosphere. One explanation is that something has filled in the craters and cracks, in a procedure called resurfacing. The most likely material for this would be liquid water, perhaps seeping up from underneath the icy crust.

One of the larger discernible craters is Pwyll. This crater seems to come from a geologically recent impact. Its estimated age is 10–100 million years since bright rays of ejected material are still radiating from the crater. The crater itself is 26 kilometers in diameter, and a darker halo with materials from underneath the surface surrounds it, which suggests that the crater is deep. However, measurements show that its crater floor is at the same level as the

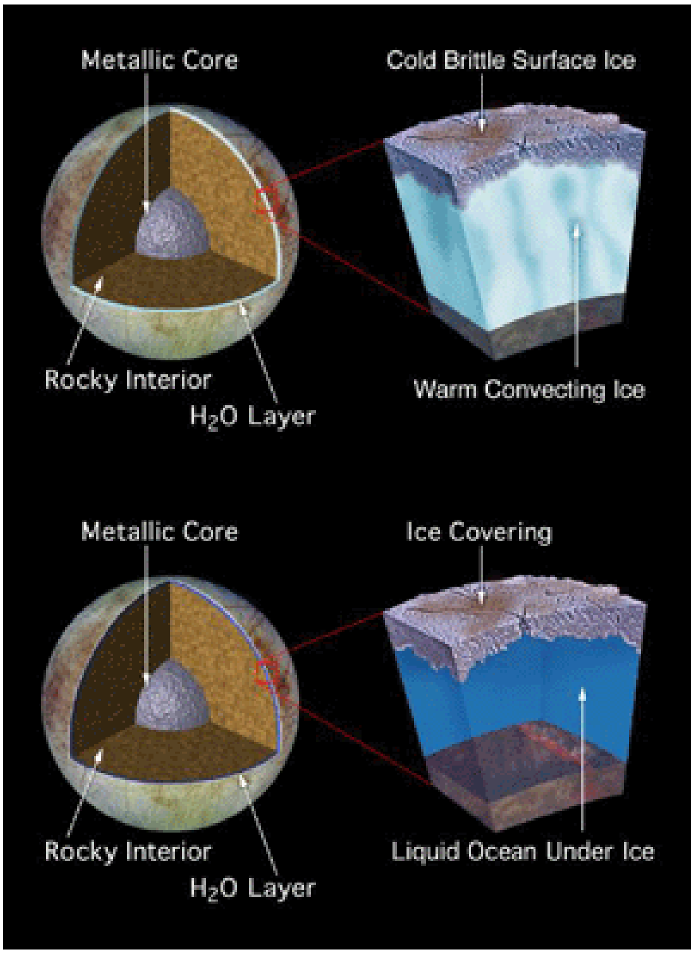

Figure 3.2: The interior of Europa, NASA.

surrounding surface. The hole that the impact created in the crust seems to have been filled in and refrozen.

Photos taken of the surface of Europa look as if they were taken over the Arctic ice sheet here on Earth, Figure 3.3. There are features similar to icebergs, which have been fractured and rotated into a so-called "chaos terrain". In between these structures there is material that looks like it has been stirred. The cracks, called "wedges", exist all over the ice and seem to have been filled in and refrozen, just like as the case with the Pwyll crater. These cracks resemble those at the mid-ocean ridges on Earth where magma is welling up to create new ocean floors due to the tectonic plate movements. This suggests that there is also some form of plate tectonic movements of the ice crust at Europa, hinting to a non-solid interior [14].

The best evidence for a possible liquid interior of water ice also comes from the Galileo spacecraft, but this time through its magnetometer measurements. This instrument recorded that Europa has a magnetic field, which is quite unusual for a moon. It was discovered also that this magnetic field changes peri-

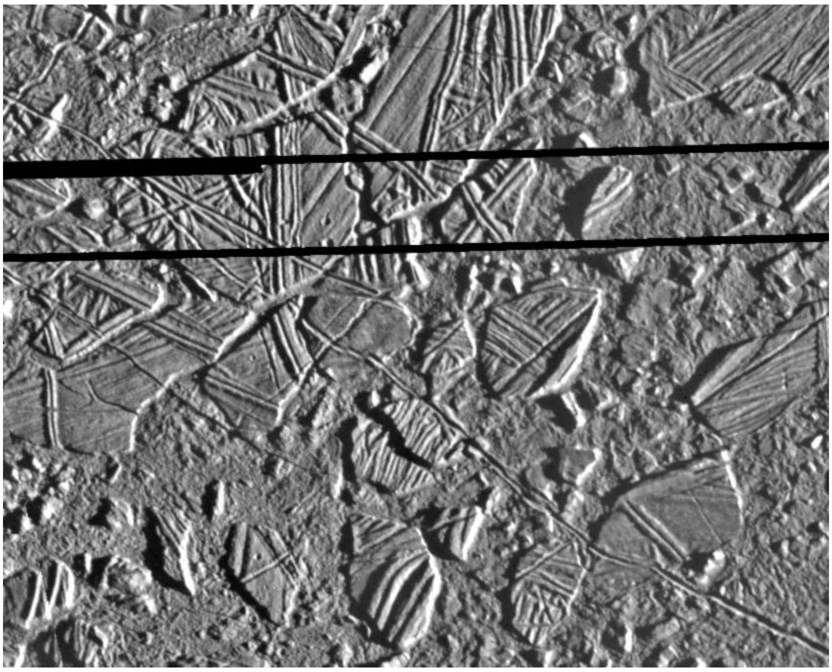

Figure 3.3: Surface of Europa, NASA JPL.

odically. Jupiter is believed to create this fluctuating magnetic field. Europa is orbiting within Jupiter's strong magnetic field, which is a bit tilted in relation to the orbit of Europa. This means that Europa moves around in this field. If there is something that can conduct electricity underneath the surface of Europa, then a magnetic field would be induced. This is much like the current created in a coil of wire that moves in relation to a magnet. The "coil" that creates an opposing magnetic field at Europa could be a global salt water ocean under the surface. Measurements indicate that it could be as salty as the oceans here on Earth, and that the water mass is twice that here on Earth.

3.1.2 Is a hydrothermal biota possible on Europa?

With enough tidal heating and any radioactive decay in the interior of Europa, a liquid ocean could exist under the icy surface. Due to the geological activity there could also exist hydrothermal vents on the bottom of the ocean floor. These vents could provide a source of energy, different chemicals and a liquid medium to create a suitable environment for life.

One of these elements in the process of life at the hydrothermal vents is oxygen. The oxygen in the water is needed in the hydrothermal vent chemistry, as shown in Equation 2.2. On Earth, the oxygen is provided to the oceans through several processes, involving the sun and the water cycle. On Europa, it would be harder for the needed oxygen to get blended down in the ice-covered oceans, but there are possibilities [15].

A first solution to this would be particles that have been accelerated to high speeds by Jupiter's magnetic field and hit the ice surface of Europa. The

addition of some ultra-violet light from the sun could break up the bonds of the ice molecules and produce molecular oxygen along with hydrogen peroxide and hydrogen. To blend this down into the ocean some additional mechanism is needed. But there are signs of such mechanisms in the pictures taken of the surface. Some sort of active ice and water mixing cycles, like ice tectonics, seems to be going on at the surface. Hot spots under the surface could be where the oxygen reaches the water underneath.

The discovery by the spacecraft Galileo of a thin oxygen atmosphere at Europa supports the idea that high-energy particles interact with the surface ice. Oxygen can also be produced through lightning and through impacts of meteorites on the surface, but no lightning has yet been detected in the thin atmosphere of Europa and impacts are too infrequent to be a good source for oxygen and mixing of the water and ice.

A second possible way for the oxygen to get into the liquid water is through the radioactive decay of potassium, which could be a part of the materials making up the core of the moon. Potassium dissolved into water could produce hydrogen and oxygen molecules of the water underneath the surface, but the question here is if there is enough potassium in the core to produce the larger amounts needed.

A more farfetched but interesting way for this oxygen to be produced and mixed into the water is through photosynthesis [15], as here on Earth. This is very unlikely though due to the weak sunlight at the moon and the very cold surface. The weak sunlight would only reach the very top of the surface ice, where the temperature is extremely low. Life as we know it requires liquid water, which would be hard at the bitter cold surface. There could, however, be microbes living underneath the ice surface. Periods in which the water melts through hot spots underneath the crust could create suitable conditions in which life could thrive for a shorter period of time. When the water refreezes life would become dormant while waiting for the surface to melt again. Some photographs show small dark patches on the surface, which could be places where warmer ice or water has reached the surface and melted the harder upper ice sheet.

Here on Earth, 400,000 year old ice samples have been collected from just some 100 of meters above Lake Vostok in Antarctica. These ice samples contained microbial life-forms such as cyan bacteria, fungi, spores and even organisms that scientists have never seen before. Lake Vostok would be a good analog environment here on Earth to Europa [22]. Current drilling methods involve drilling oils and chemicals that will contaminate the lake underneath the ice, which has been a closed environment for around one million years. Other drilling methods are being developed, in order to minimize the risk of contaminating the lake water.

3.2 Enceladus

Resent remarkable discoveries made by the Cassini spacecraft show geyser-like features on Saturn's small moon Enceladus [13, 16], as shown in Figure 3.4. Not only do images show the geysers, but temperature readings from the spacecraft also reveal that these geysers are emanating from warm spots at a feature on the surface called the "tiger stripes". It is thought that temperatures above freezing can exist just some ten meters under the frozen surface, creating pockets of liquid

water. The water could perhaps even be heated to near the boiling point by magma in a hot spot in the southern polar region of Enceladus, Figure 3.5.

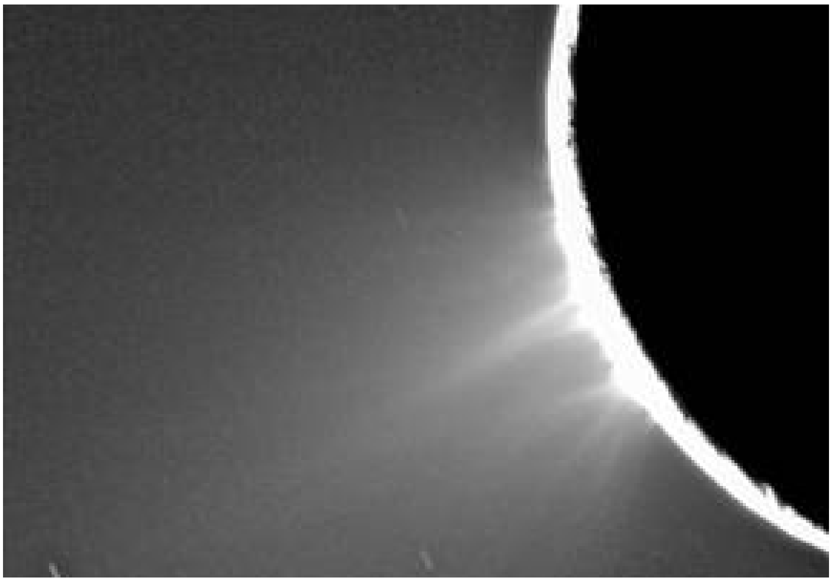

Figure 3.4: Backlit geysers on Enceladus, NASA/JPL/SSI.

The water plumes are emanating from the southern polar region on the moon and reach a height of hundreds of kilometers. These plumes contain several organic materials, which mean that life could exist by hydrothermally similar features under the crust of the moon, in a liquid and nutrient-rich environment.

These geyser plumes turn into ice crystals, coming down back to the surface as snow. Some of these crystals manage to escape the gravity of the moon and become part of the thin E-ring of Saturn. The areas around the tiger stripes on the surface lack craters, indicating a young surface.

The moon Enceladus is a very small body. It has a diameter of 500 km, about seven times smaller than the diameter of Earth's moon. It's a mystery how liquid water can exist on such a small and cold moon. Nevertheless, this discovery has broadened the diversity and possibilities for hydrothermal vent-like systems to exist beyond Earth in the solar system. The next close approach for the Cassini probe to Enceladus is scheduled to 2008, but the Cassini team now looks into changing the orbit for an earlier return.

3.3 Future exploration of Europa

In the Search for Origins of Life program at NASA, technology is developed for high-level discovery missions. One of these is a probe to be sent to Europa. This probe would penetrate the icy crust of the moon in search for hydrothermal vents in an underlying water ocean, Figure 3.6.

However, before any lander or probe is sent to the moon, more analyze and reconnaissance missions are needed. The state of the subsurface of the moon needs to be established, i.e. that there really is a liquid ocean underneath the

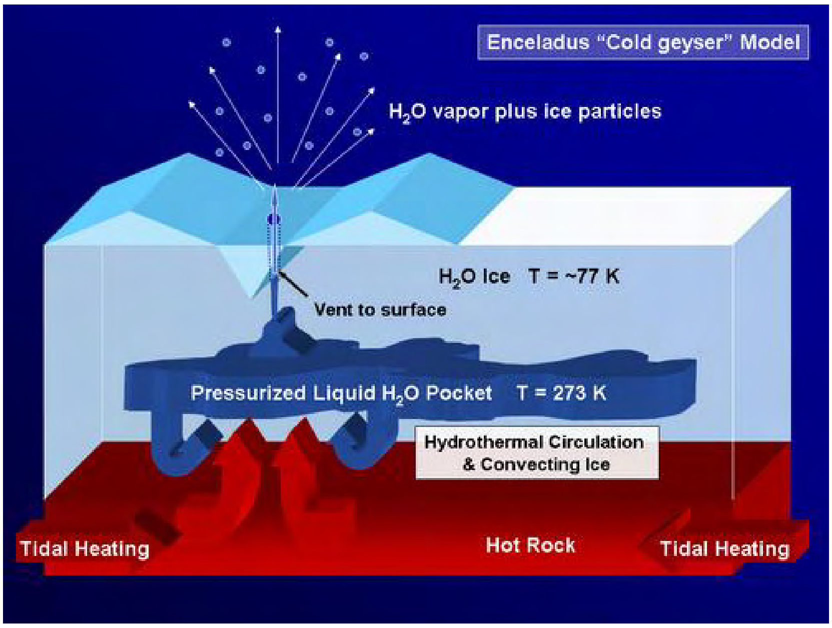

Figure 3.5: A possible eruption cycle of Enceladus, [16].

ice surface.

The Europa Orbiter was suggested as a first step to help solve the question of Europa's interior, but this project was removed from NASA's FY2003 budget to Congress [21]. A spacecraft such as this needs to be sent to Europa carrying with it instruments to make better measurements of the moon's characteristics. Deep penetrating radar would be well-suited for looking through the ice, and this kind of instrument was used to discover Lake Vostok beneath five kilometers of ice in the Antarctic. Using an instrument such as this, the thickness and possible weak spots in the ice could be detected, and a location established to which a future ice-penetrating vehicle could be sent.

An altimeter could also be used to detect if Europa has a liquid interior. Due to the tidal forces acting on the moon, the surface would bulge like the oceans do on Earth due to the gravity. These bulges are estimated to be in the order of one meter if there is a solid interior, but up to about 40 meters if there is water in between the core and the ice crust.

Missions proposed to follow up on a scouting Europa orbiter are the Europa Ice Clipper [12] and the Europa Ocean Explorer, Icepick [23]. The Europa Ice Clipper mission would be a flyby mission to explore the ice of the moon by releasing a hollow copper sphere down to the surface. Upon impact materials from the surface would be ejected and the spacecraft would be diverted to fly through this debris and collect samples.

The European Ocean Explorer, also called Icepick, would land on the surface of the moon. From here it could send out a cryobot, which is a pencil-shaped probe using heat to melt its way down through the ice. This probe would take measurements of the ice along the way down, and when reaching the bottom of the ice cover, release different devices for making measurements. One of

Figure 3.6: A hydrobot searching a Europan ocean for hydrothermal vents, NASA /JPL/Caltech.

these devices could stay in the boundary region of the water and ice and make measurements. Another sinkable device could make its way down to the bottom, and a third type could be a hydrobot, a robotic vehicle that could move freely in the ocean water.

Chapter 4

The hydrothermal vent bio-sampler

The missions stated in the previous chapter are yet far away in to the future. Something closer in time is the Hydrothermal Vent Bio-sampler (HVB) under developing and testing at NASA JPL. This is an instrument to take samples and search for extreme microbial life in deep-sea hydrothermal vents.

4.1 Purpose of the HVB project

The purpose of the HVB project is to take in-situ measurements and to collect pristine samples of the vent fluids emanating from the hydrothermal vents. Analysis in laboratories will then be performed on the samples the instrument has taken, looking for signatures of life. It will also be a test of the techniques needed for a probing and sampling device, to be used both here on Earth and on other planetary bodies in the solar system, such as the Galilean moon Europa.

The HVB is founded by the Astrobiology Science and Technology Instrument Development program of NASA. To this project, technical inputs are provided also by the Monterey Bay Aquatic Research Institute (MBARI), Scripps Institution of Oceanography (SIO) and Woods Hole Oceanographic Institute (WHOI). The initial goals for the HVB system can be found in Table 4.1.

Samples have been taken from hydrothermal vents before, but these have not been proven pristine, and the in-situ bioassay techniques used had not been validated before [2]. The surrounding water of a hydrothermal vent plume is a region teeming with microbes and particles, due to the nutrient-rich environment created when the hot vent water is mixed with the cold sea water. It is therefore important for the sampling instrument to be designed and built with this in mind in order to prevent any contaminates reaching the samples from within the vent plume.

The water that is to be probed can also be very warm, up to 400 °C, and any microbes found in this extremely warm environment are expected to be in a dormant state and to have a very low biomass. The sampling system therefore needs to be able to take samples of the vent water without contamination of the surrounding environment and to filter large volumes in order to collect enough biomass.

System specific	Goal
Time to sample	≈ 5 minutes
Volume to sample	20 liters
Filter pore size	1 mm, 100 μm, 100 nm
Mass	< 10 kg
Power	10 W
Max depth and temp. Rating 6.5 km	450 bar, 400 °C
Volume	12 in long (0.3 m), Ø6 in (0.15 m)
Tubing length	6 feet (1.8 m)
Number of filter stages	3
Time to clean (redeploy)	30 minutes
Usage	deplyment from manned/ unmanned submersible
Actuator	DC motor, precious metal brushes fluorinert filled
Communication	inductively coupled link or RS-232
Sensing	real-time temp and pressure measurement
Control	sampling on/off, filter line selection
Maintain	85 °C
Operating Time	5 hours
Pressure Measurement	0,03 mbar accuracy, 100 m, 5 s
Temperature Measurement	1 K accuracy, 1 s/sample
Development cost	less than $50,000 US
Development time	36 months

Table 4.1: Initial goals of final version of HVB, set in 2004 [2].

The HVB system will be able to probe, collect and filter a large amount of hydrothermal vent water, and to bring these samples up to the surface without any contamination from the surroundings. These samples will then be analyzed in place or taken to a laboratory, to look for microbial life. A discovery of microbes living in the superheated water from the vent plumes would probably set a new boundary of the extreme life as we currently know it.

4.2 System architecture and operations

The HVB instrument will consist of a core unit with a sampling nozzle attached to it through a flexible hose. The operation of the system is similar to the operation of a normal vacuum cleaner, pulling air and dust in through a nozzle to a core unit where a filter is used to trap the dust, and then vents the air back out from the system. For the HVB however, the "air" is the superheated vent water, and the dust is the particles and any microbes that can be found in the water.

The overall system architecture schematics and operation of the HVB can be seen in Figure 4.1. The HVB system will be carried by a submersible vehicle, such as the Alvin or Shinkai 6500, deployed from a research vessel situated above

the area of interest. The system will be fixed to the submersible in its cargo bay. Before the system is deployed the tubes and filters will be primed with a sterile liquid to avoid any implosions due to trapped air in the system, and any contaminations in the filters. Water will be pumped through the bypass pipes, parallel to the filter assembly, in order to verify the operation of the system before descending to the hydrothermal vent. Running water through the system will also flush out any previous contamination, a technique which will be used before accessing a new filter line.

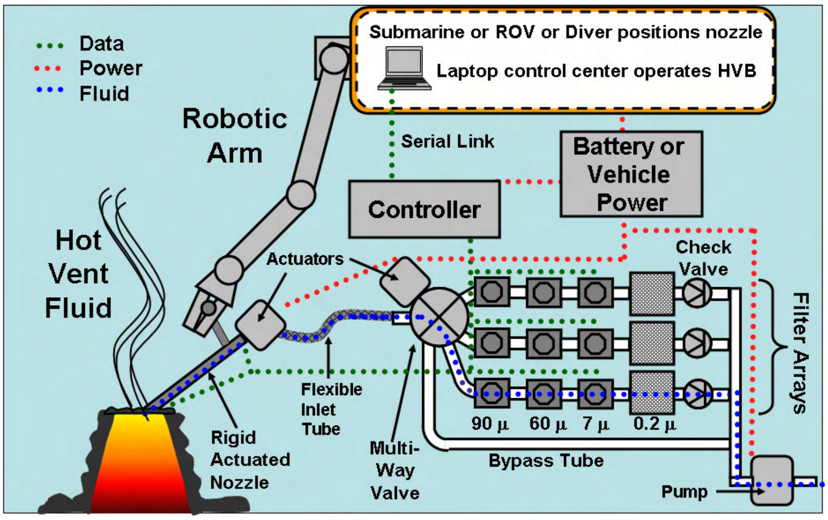

Figure 4.1: The system and operation of the HVB, NASA JPL.

When the submersible reaches the target hydrothermal vent it will hover by the vent while the instrument is under operation. The intake nozzle of the HVB will be positioned in the deep-sea hydrothermal vent plume by the submersibles robotic arm. By visual, temperature and pressure readings, the correct position of the nozzle will be verified before the intake is opened, avoiding any contamination by the surrounding water. The vent water will be pumped through the nozzle to the main body of the system, where a series of filters will collect any particles in the water. Along the way through the pipes that the vent water passes, there will be temperature sensors, as well as a flow meter, to record the sampling conditions. Real-time data will be sent from the instrument to the submersible and/or up to the research vessel at the surface. From here the progress will be followed and the system operated. It is estimated that 10–20 liters of vent water will have to be filtered in order to collect enough biomass in the pores of the filters. The instrument will have three arrays of filters, thus being able to take three different samples during one deployment.

When enough vent water has been sampled and filtered in the system, the submersible will return to the surface and the research vessel. Each filter-line will be closed off from the surrounding by the four-way valve and a check valve, to prevent any contamination of the samples from the surroundings. The filters with the samples will then be removed from the system and either processed in place or stored for analysis on mainland.

4.3 Environmental requirements

The instrument must comply with the design constraints and requirements imposed by the extreme and hostile environment found at these hydrothermal vents.

The HVB is built for operations under water, meaning that all electronics and mechanical parts must be protected against any damages, such as short-circuits or corrosion by the saline seawater.

The mass of the system needs to be kept low, since a too massive system cannot be carried by a smaller submersible. Though the system will be lighter in the water, the current design is still too heavy. By attaching floats on the system, neutral buoyancy can be achieved but the mass of the system will still pose maneuverability problems.

The pressure will be several hundred times greater at the vents than at the surface. Above the sea surface the pressure is one atmosphere, or 14.7 PSI. The pressure increases as one descends below the surface with one atmosphere for every 10 meters. This means that at the average depth where the hydrothermal vents can be found, at 2500 meters, the pressure will be 251 atmospheres, equal to 3690 PSI. The pressure requirement is the biggest challenge and puts the main constraint on the components and design of the system.

In order for an instrument to qualify for being attached and operated by the exploration vehicle Alvin, it must be able to withstand a series of pressure tests of 10,000 PSI, equaling a depth of 6800 meters below the surface. Therefore the system needs to be tested and verified to withstand this pressure.

The HVB also faces a challenge due to the extreme temperature. The water from the vents can be up to 400 °C with the surrounding water being just 2 °C. When choosing the materials to be used, especially in the nozzle, one must have this in mind. The closer the different components in the system are to the nozzle and the pipes, the warmer their operation environment is going to be.

The material must not only be able to handle the extreme temperature, but must also be able to withstand the strong corrosive chemicals, like the sulfuric acid, found in the waters around the vents.

The pristine samples must be taken so as to ensure that any microbes found in the filters truly come from the area of interest within the vent plume. The colder water surrounding the vent is teeming with microbes living of the nutrients provided by the vent. The water expelled through the vent will emanate with a high enough flow to keep these ambient microbes away from the inside of the plume, and thus any microbes that could be found in the superheated vent water plume should be native to the hot water environment. They might, however, be in a dormant condition and have a low biomass due to the high temperature. Therefore a large amount of water should be filtered to increase the amount of biomass the filters can collect.

Chapter 5

HVB subsystems

This section describes each of the subsystems making up the HVB, Figure 5.1. All components are mounted on a custom made frame, making the system more manageable for transportation and tests. One side of the plate, called the front side, holds the main electronics box, pressure sensor and pressure compensator. On the other side, the back side of the system, the piping with the filter assembly is placed. Due to the fact that this system is still in its development phase, only one filter line along with the bypass-line has been installed. The final version will contain the other two filter lines as well. Each of the subsystems is further described in their respective section below.

5.1 Structure and Mechanisms

The structure is the skeleton of the HVB. A metal frame acts as the center of the system to which all other units are attached. The materials used need to withstand the extreme environment encountered by the vents at the bottom of the ocean. The system needs to be rigid and robust but in the same time be light for easy handling. The material used for the frame of the system is aluminum, which is a light metal but also makes a robust construction. Aluminum is also easy to work with, which is a very important factor in the development phase of the project with continuous modifications and changes. The components used in the current HVB system are listed in Table 5.1.

5.1.1 Electronics box

As is well known, water and electronics do not mix well. The HVB needs electronic circuits to operate and these will have to be isolated and protected from the ocean water during deployment. This is done by placing the electronics and other water sensitive components in a water-tight box, Figure 5.2. This will solve the problem of the water coming into contact with the electronics, but will on the other hand create a new one. When the sealed-off box, containing one atmosphere of pressure, is submerged in water the pressure outside the box will be larger than the pressure inside. The further the box is submerged, the higher the pressure difference. When it becomes too high the box will implode, not only destroying the system but also creating a great danger for the submersible

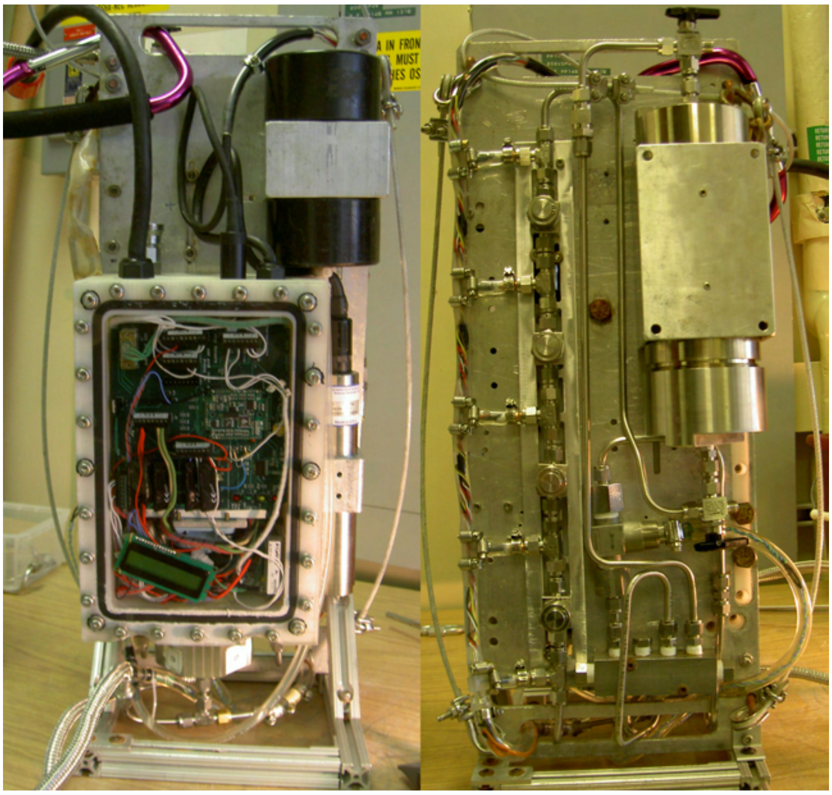

Figure 5.1: Front and back view of the HVB instrument.

carrying the system. In order for the box to withstand a stronger force, a stronger material and construction of the box are needed. This will, in turn, result in a larger, heavier and more expensive box. In order for the box to withstand the extreme pressures at the required depth, the construction might turn out to be too heavy and expensive.

An alternative solution to handle the pressure problem is to have the inside pressure equal the outside pressure. In this case there will be no pressure difference and the box can be built of lighter and cheaper materials. The pressure bearing requirement is thus moved from the electronics box itself to the components inside. This latter solution was chosen for the HVB.

A thermoplastic material, Delrin, was chosen to make up the box. It is an acetal resin engineering plastic, invented and now sold by DuPont as a metal substitute. This is a lightweight and wear-resistant plastic, which can withstand temperatures of over 90 °C. This is estimated to be enough thermally-tolerant since the box will be placed in the cold water by the submersible and not in the actual superheated vent plume itself.

The lid of the box is made of lexan, manufactured by General Electric Plastics. Lexan is a highly durable polycarbonate resin thermoplastic, similar to polymethyl methacrylate, also known as Plexiglas, but more durable. It is often used as a replacement to glass where the strength of the material justifies the higher price, such as in aircraft windscreens and bullet resistant glass. Since

Component	Model	Manufacturer
DC brushless pump motor	380A	Micropump
DC/DC Converter	UCP-12/5-D24	Datel
Depth Sensor	SBE 50	Seabird Electronics Semiconductor
Flow Meter	FTB-9501	Omega Engineering
Flow Meter Amplifiter	FLSC-AMP/AMP8	Omega Engineering
Filter	SS-4TF-LE	Swagelok
Filter	Custom made	Mott Corporation
Four-way valve	SS-45ZF8-ND	Swagelok
Four-way valve servo	SSPS-105	Tonegawa Seiko
LCD	BPK-216N	Scott Edwards Electronics
Motherboard	HVB v1.0	JPL
Nozzle servo	HT17-075	Ultra Motion LLC
Pump	GB-P35	Micropump
SS Poppet Check Valve	SS-CHS4-KZ-1/3	Swagelok
Thermocouples	HKMTSS-040U-12	Omega Engineering
Thermometers	DS1822	Dallas/Maxim

Table 5.1: Components of the HVB system.

lexan is transparent, the system can be supervised visually while sealed up. The cover is tightened to the box with 28 screws and combined with a rubber O-ring, to make the system water-tight.

In order to keep the internal pressure equal to the external pressure, the Delrin box containing the electronics is filled with a non-conducting liquid. This is done through two valves, one at the top of the box and the other at the bottom. In addition, a pressure-compensating bladder, filled with the non-conductive liquid, is placed outside the box and connected through a hose. The liquid chosen is Fluorinert, sold by 3M. Fluorinert is an inert perfluorocarbon non-conducting liquid and contains no chlorine atoms, thus poses no threat to the ozone layer depletion. It is, however, recommended to be handled carefully since it has a long atmospheric lifetime. Fluorinert is electrically insulating and mainly used as a cooling liquid for electronics. In this project it is used as the box-filling liquid and pressure compensator. Normal water could have been used as a pressure compensator, if it would not have been for the electronics. Oil is another possible liquid, but has the downside of leaving an oily residue on all surfaces when drained.

In addition to various electronic components, the peristaltic pump, the servo to operate the four-way valve and a flow sensor are also placed inside the Delrin box. These units are described in their own sections below. Pipes and cables are entering and exiting the box to connect the different units. These connections to the box are carefully sealed not to cause any leakage pathways.

Due to the increased pressure inside the box when the system is descending into the deep, all components inside must be able to handle this pressure as well. This means that there cannot be any other airtight containers, such as the housing of a motor or that of an electrolytic capacitor, which could implode. Either these airtight parts will have to be punctuated and filled with Fluorinert

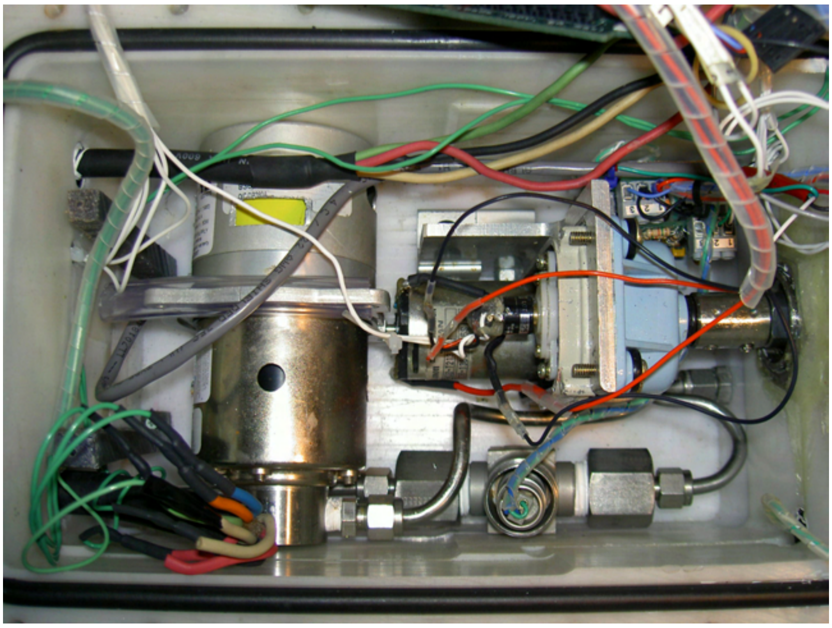

Figure 5.2: Inside view of the Delrin electronics box, with the covering motherboard removed.

or replaced with another more pressure-resistant similar component.

5.1.2 Pressure compensator

A bladder with extra Fluorinert liquid is mounted on the outside of the box and connected through a hose to the Delrin box, Figure 5.3. As the pressure increases around the box, the bladder will experience the same pressure increase and through the hose pressure compensate the inside of the Delrin box. Since liquids are very incompressible substances, only a small amount of Fluorinert will be needed in order to increase the pressure inside the box by a substantial amount. If air would have been used, a large amount would have to be pumped in to or out of the box in order to compensate for pressure.

5.1.3 Piping

A schematic of the filter piping line and the interfacing to its different units can be seen in Figure 5.4. The vent water entering the nozzle is led through a flexible steel hose to the main body of the HVB at the submersible vehicle. Here the water first passes through the four-way valve. This valve is operated by the command center, and its status depends on whether the water is to be flushed through the bypass line or through one of the filter lines. The bypass line and the filter lines are connected in parallel from the four-way valve and come together again at a manifold to form a single pipe just before entering the pump. The bypass line is used to flush water through the system to verify the operation before deployment and in between the samples to rinse the pipes. Since this line does not have the flow restricting filters, a higher flow rate will

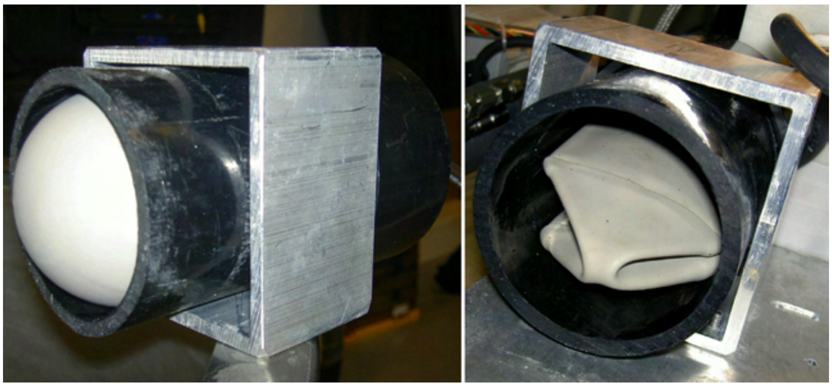

Figure 5.3: The pressure compensating bladder, full to the left and empty to the right.

be achieved, which will help to flush any microbes or other contaminates out of the system.

In the case of the filter array, there is a check valve positioned in the end of each line before the manifold. These check valves will prohibit any water from entering from behind and contaminating the filter arrays. The front end is closed off by the four-way valve. The pipes and filter in between these can therefore be closed off from the surroundings, ensuring that no contamination will occur.

After the manifold the pump is attached in line to pull the water through the system. The pump will be less efficient pulling water through the pipes than pushing it, but having it in the end will avoid any contamination and the need to sterilize the pump. A pump in the beginning of the system would also need to handle higher water temperatures.

The last piece of the filter array is the flow meter, situated behind the pump to monitor the flow through the system before the sampled water is flushed out from the system. During the development phase an extra flow meter is placed in the bypass line to aid in the performed tests. This flow meter will be removed in the final design.

The pipes in the system are made of stainless steel with a diameter of 0.250 x 0.035 inches (6.35 x 0.889 mm), the first being the outer, and the latter the inner diameter. The connections between the different pipes and the other units are by Swagelok fittings, a nut-ferrule set providing with a tight interface, Figure 5.5. The pipes are bent and formed into suitable shapes and have their fittings attached. All is manufactured and shaped in the laboratory depending on the needed design. Due to the iterative process of developing the HVB system, where many components have been moved, replaced or modified, the making and fitting of pipes have been a major part of the mechanical work.

The nozzle intake must be able to withstand the high temperatures in the hydrothermal vent water plume, up to 400 °C. The superheated water will cool down as it travels along the pipes, leading the sampled water to the sensors and filters, which also must be able to resist a high temperature. Materials such as aluminum, stainless steel and the heat resistant polymer PVC, Polyvinyl chloride, are used for this.

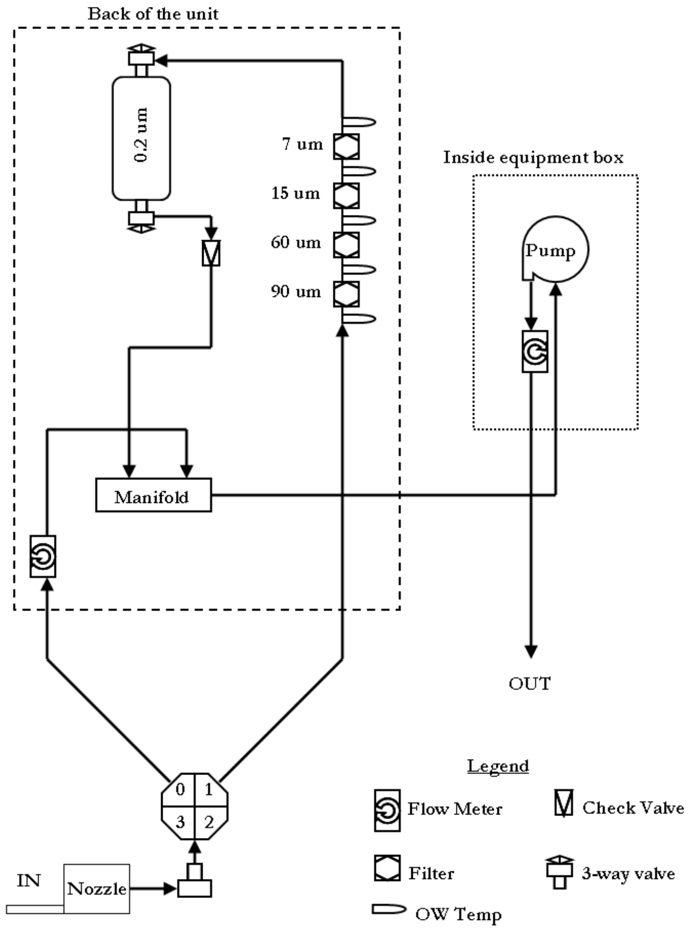

Figure 5.4: Filter line connection.

5.1.4 Filters

In order to sample the microbes of interest from the vent water, a series of filters are used. A filter line is composed of five different individual filters in a decreasing pore size order of 90, 60, 15, 7 and 0.2 μm. The first four filters are made by Swagelok (SS-4TF-LE) and are used to filter out any larger particles and materials from the vent water. The last filter is a custom-made 0.2 μm filter by Mott Corporation, shaped as a cylinder. It has a larger surface area than the other four filters in line, and is to trap and collect any microbes from the sampled vent water. The final system is to have three identical and parallel filter-lines, enabling the HVB to take three different samples during one deployment. However, during the development phase only one of these filter-lines is attached, making the current system consist of one bypass-line and one filter-line.

An earlier version of the 0.2 μm filter was tested at Woods Hole Oceanographic Institute (WHOI) and it did not manage to withstand the high pressure, Figure 5.6. The probable cause of this malfunction was air still trapped inside the unit, which caused it to implode during the test. The new filter by Mott

Figure 5.5: Nut-ferrule Swagelok fitting.

Corporation is larger and more robust than the older one, to make sure it will not fail again during testing. A downside of this is that it weighs 9.5 kg, a major part of the total mass of the HVB system. For the final system a lighter version must be used. With correct placing of the filter and the right priming procedures of the pipe system, any air should be excluded and a less massive filter could be used.

5.1.5 Tethers and cables

This main tether will be used only during the testing phase. For a real deployment of the finished system, the HVB will be connected through a short interface to the submersible carrying it. From there, the submersibles communication and power resources will be used.

The main tether was manufactured by Falmat in San Diego. It is 150 feet (47 m) long and includes both power and signal lines for communication and control, Figure 5.7. An earlier version of the main tether had problems with system resets when running heavier loads. This was found to be caused by noise introduced to the ATN line in the RS-232 serial interface. This line is used as a system reset. The problem was temporarily solved by additional shielding of the tether, but due to wear and tear a new cable had to be manufactured.

The minor cables used in the HVB system were manufactured in the lab and consist of wires run inside PVC tubes. These tubes are filled with Fluorinert or epoxy in order to minimize the amount of trapped air, and thus avoiding any major compressions.

The interfaces of the cables are made by Impulse, a San Diego company specializing in cables and connectors for marine applications. These rubber connectors ensure a tight seal, keeping the surrounding water away from the connector pins.

Figure 5.6: Old imploded 0.2 μm filter with the new version in the background.

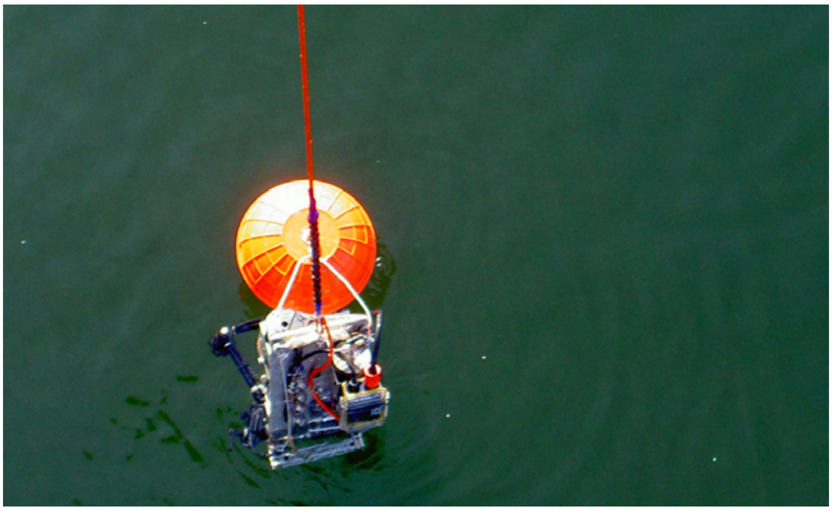

Figure 5.7: HVB deployed off a pier with the orange tether and having a buoy attached.

5.2 Electronics and Control

A schematic of the electronic components in the HVB system is shown in Figure 5.8. The electronic components needed to run and control the HVB system are enclosed inside protective boxes, filled with a non-conductive material, in order to isolate them from the surrounding water. The boxes have an internal pressure equal to that of the outside, in order to save mass of the boxes. This transfers the high pressure problem from the boxes to the components inside. Thus all electronic components used in the system will have to be able to withstand the high pressure at the depths where the deep-sea hydrothermal vents can be found.

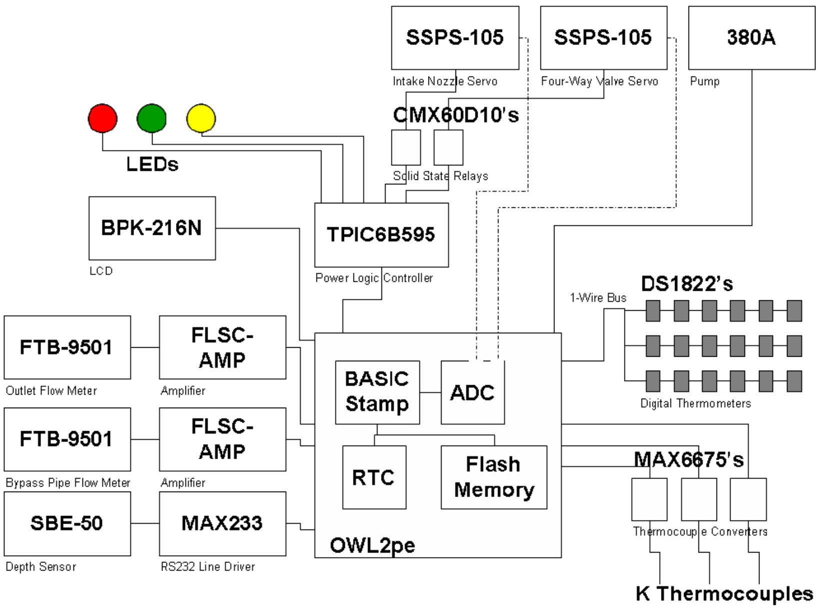

Figure 5.8: Schematic of the electronics system [10].

The Capacitors

For the individual electronic components, the aluminum electrolytic capacitors commonly used in any electronic circuits will not be suitable for the HVB. Under the higher pressures experienced by the HVB the capacitor structure implodes, destroying the component and endangering the rest of the system, Figure 5.9.

To avoid this, capacitors built with the chemical element tantalum replace the aluminum electrolytic capacitors. Tantalum has atomic number 73 and this transition metal is a dense, ductile and hard material. It is also very resistant to corrosion. A high capacitance can be achieved in a small volume thanks to its dielectric layer, a protective oxide formed by the tantalum material, which can be made very thin. The tantalum capacitors can therefore better survive the higher pressures than the aluminum capacitors. The tantalum capacitors are, however, more expensive and are usually not built to handle the higher voltages required in this project.

Figure 5.9: Pressure effects on an aluminum electrolytic capacitor.

5.2.1 Microprocessor

The brain of any computerized system is the microprocessor. The HVB uses the OWL2pe from EME Systems, Figure 5.10. The OWL2pe is built using the core of the BASIC Stamp 2pe from Parallax Inc., but has some extra features enhancing its data logging ability. The components making up the OWL2pe can be seen in Figure 5.10 and Table 5.2 [4].

Number	Component	Description
1)	BASIC Stamp 2pe core	8 MHz Ubicom SX48
2)	24WC256	32 kbyte EEPROM
3)	DS1307	Real Time Clock
4)	TLC2543	11 channels, 12 bit ADC
5)	LT1790	4.096 V reference for ADC
6)	LM50	Temperature sensor
7)	AT45DB041	512 kB flash memory for data logging
8)	LT17613	3.3 V regulator
9)	LT1521-5	5 V regulator
10)	LT1521	Adjustable regulator
11)	UN2111	Transistors for RS-232
12)	330 Ω network	I/O protection

Table 5.2: OWL2pe components, see Figure 5.10

The Parallax Stamp processors are very popular because they are easy to use. This enables projects to move quickly from a concept to its implementation, and changes are easily made without having to take the project back to the drawing board. External units are also easily interfaced and controlled by the Stamp. In order to program the Stamp, the specially developed PBASIC (Parallax Beginner's All-purpose Symbolic Instruction Code) language, which is further described in its own section below.

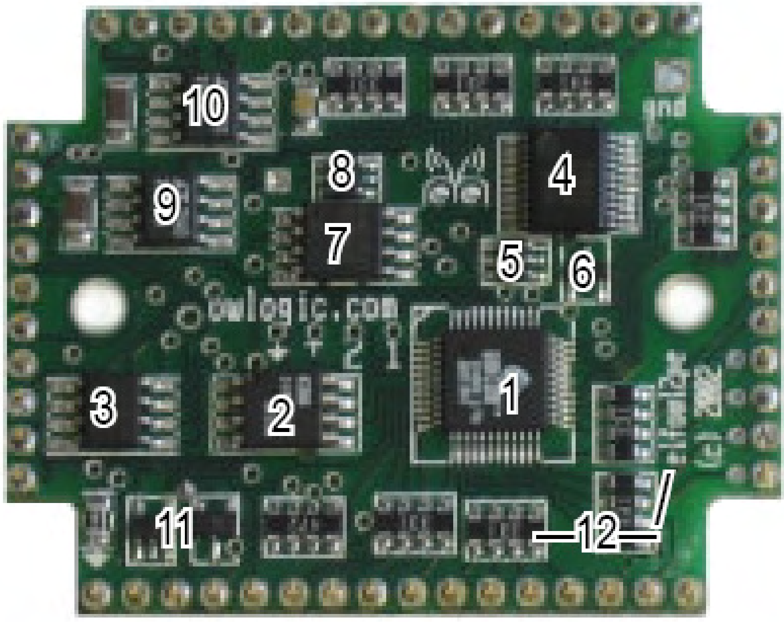

Figure 5.10: OWL2pe, EME Systems, see Table 5.2.

Core unit

The characteristics of the Stamp 2pe can be found in Table 5.3. The core of the Stamp 2pe is an Ubicom SX48 processor with a speed of 8 MHz. This is low compared to the rest of the Parallax BASIC Stamp 2 family, but the upside to a slower processor is that the power consumption is also lower. This is well suited for a logging system, which most likely will be running on battery power. The BS2pe has 32k EEPROM, which is twice as much as the rest of the BS2 family. As with the other Stamps, 16 kB is divided in eight databanks of 2 kB each, and is dedicated as program memory. The rest of the 16 kB, also in banks of 2x8 kB, can be used as additional non-volatile data storage.

Processor Speed	8 MHz Turbo
Program Execution Speed	≈ 6000 instructions/s
RAM	12 bytes I/O
	26 bytes variables
Scratchpad RAM	128 bytes
EEPROM (program and data)	16 x 2kB
	≈ 4000 instructions
I/O pins	16 + 2 dedicated serial

Table 5.3: Stamp 2pe specifications, Parallax.com.

The BASIC Stamp 2pe has 32 ports for general I/O interfaces. These ports are divided into 2 groups of 16 ports each, called the main and the auxiliary ports. 12 of these ports are used by the OWL2pe internally to interface to var-

ious components, such as the real time clock (RTC), analog to digital converter (ADC) and voltage regulators. The remaining 20 I/O ports are available to be used as interfaces to other external units, 16 main and 4 auxiliary, Table 5.4. In the BASIC program the MAINIO and AUXIO commands must be used to switch between these ports, since out of the 12 bytes of I/O memory, only 4 bytes ($4^2 = 16$) are used for addressing the current ports.

Pin	Connection
P0	Nozzle servo control
P1	Pump control
P2	Flow meter 2 data
P3	Nozzle servo control
P4	Flow meter 1 data
P5	Four-way valve control
P6	LCD serial output
P7	One-wire bus
P8	Thermocouple converter clock
P9	Thermocouple converter chip select
P10	Power logic controller data
P11	Power logic controller shift-register clock
P12	Power logic controller register clock
P13	Thermocouple 1 data
P14	Thermocouple 2 data
P15	Thermocouple 3 data
X0	Depth sensor serial data out
X1	Depth sensor serial data in
X2	MAX233 RS-232 driver serial out
X3	MAX233 RS-232 driver serial in

Table 5.4: I/O ports for external devices.

Real-Time Clock

The real-time clock (DS1307) provides to the HVB with an accurate time stamp for the measured data. This chip is powered by its own lithium battery, which has a life-time of approximately 10 years. The data are sent in BCD format to the microprocessor, which can then read the data as binary encoded, and output it in hexadecimal form. The real-time clock also has 56 bytes of RAM backed up by the battery, which holds important data logging pointers needed to survive a main power outage. This RAM can be used also to store other user-defined variables, critical to survive a power outage.

The real-time clock uses the I^2C bus protocol, supported by the Stamp2pe, to send the data. I^2C is an acronym for Inter-Integrated Circuit and is a serial bus invented by Philips for control electronics. I^2C uses two bi-directional lines, one for sending data and the other for sending a clock signal. I^2C uses a 7 bit address, of which 16 are reserved addresses, so up to 112 individual units can use the same bus.

Scratchpad RAM

The Stamp uses 128 bytes of Scratchpad RAM as a temporary storage place for data. This storage is not addressable by variable names, but with the address numbers in blocks of one byte each. The advantage of the Scratchpad RAM over EEPROM is that it is faster to store and retrieve data this way. Scratchpad RAM is a volatile memory and thus does not keep its data when power is switched off. This memory is usually used, as its name implies, as a scratchpad for taking quick notes and then storing the data in a non-volatile memory at a later time.

Flash Memory

For long-time storage of the data, a non-volatile AT45DB041 memory module is used. This IC provides 540,672 bytes, over half a megabyte, of flash memory, and also has 528 bytes of RAM used as a read and write buffer. Flash memory is a bit different from EEPROM in the way that data have to be written, one page at a time. The flash memory is arranged in 2048 pages of 264 bytes per page, and the RAM in 2 pages of 264 bytes each. The RAM buffers are used for passing data in and out of the flash memory. When data are not stored or read out from the flash memory, the RAM buffers are available as additional general purpose storage.

Analog to Digital Conversion

Microprocessors can read only digital values, 1s and 0s, such as from a switch with an on and an off state. But in many applications there are states in between on and off. For example a door can be open or closed, but also in a state in between. In order to detect this in-between state a potentiometer can be used. It is a variable resistor, and as the resistance is varied, through a mechanical action such as the state of a door, the voltage over this resistor will vary as well. An analog-to-digital converter (ADC) will read this voltage and convert it to a digital number. An 8-bit potentiometer would convert 0 V to number 0 and 5 V to number 255 (2^8=256). Each step of the number would represent a voltage of about 0.02 (5/256) volts, which is called the resolution of the ADC.

On the OWL2pe there is an ADC, which provides 11 analog-to-digital conversion channels. The converter is 12 bits and uses a reference voltage of 4.096 VDC from the OWL2pe, resulting in a resolution of 1 mV ($4.096/2^{12}$). The two last channels are used by the input voltage and the onboard temperature sensor, leaving the other nine for the HVB system, Table 5.5. The HVB uses only the first two channels for the feedback from the two motors. The feedback consists of rotational potentiometers, which give a value from 0 to 4095 depending on the position of the motor which they are connected to. The communication with the ADC is done through a Serial Peripheral Interface (SPI). SPI is a loose but cheap standard for controlling a serial interface. It is cheap in the sense that it does not take up much space in an IC.

5.2.2 Motherboard

If the microprocessor is the brain, then the motherboard is the spine of the system, Figure 5.11. The motherboard is placed in the electronics box and

Channel	Connection
0	Four-way valve potentiometer
1	Nozzle actuator potentiometer
2-8	Unused
9	Onboard temperature
10	Input voltage

Table 5.5: ADC channel assignments.

acts as the interface unit between the microprocessor and the external units, through ports and connectors. There are also additional components mounted on the motherboard to complement and support the microprocessors operation and functionality, Table 5.6. The motherboard for the HVB was created in an early state of the project, and as the system evolved changes have been made, where some components have become obsolete and others added to the board.

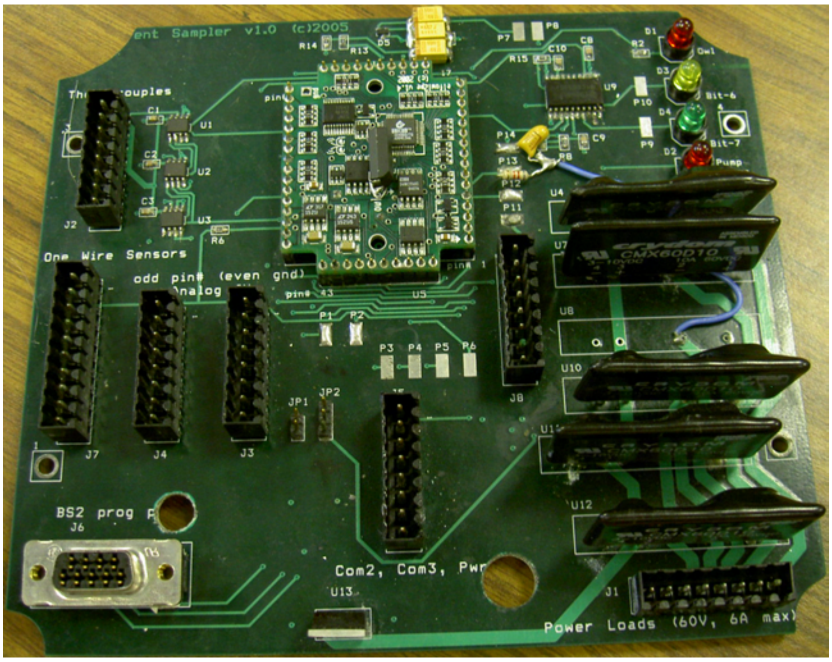

Figure 5.11: The motherboard.

There are four LEDs placed on the motherboard to indicate the status of the system and these are used for debugging and testing.

The most notable feature of the motherboard is the black solid state relays. These are placed on the motherboard in order for the microprocessor to control high power units, such as the motors and the pump. The solid state relays are further described below in the power section.

Component	Model	Manufacturer
Microcontroller	OWL2pe 1.4	EME Systems
Power Logic Controller	TPIC6B595	Texas Instrument
Solid State Relays	CMX60D10	Crydom
RS-232 Driver	MAX233AEWP Semiconductor	Dallas/Maxim
Thermocouple Converters	MAX6675	Maxim Integrated Products
5V regulator	LM7805C	National Semiconductor
Status LEDs		
External connectors		

Table 5.6: Motherboard components.

External connectors

The connectors named J1, J2, J3, J4, J5, J7 and J8 provide an easier accessible connection to the pins of the microprocessor. Each connector has room for eight connections, except for J7, with room for ten. Some of these connections are used by two or more wires at the same time in the current system.

5.2.3 Power

There are many high-power consuming devices in the HVB system. Table 5.7 shows the components and what voltage, current and power requirement they have. The HVB is supplied with at least 24 VDC and 3 A from an external power supply through the main tether. The pump is powered directly from the power supply, while the other subsystems are using down-converted power. 12 VDC is acquired with a converter, which is also connected directly to the external power supply, like the pump. The 12 VDC is, in turn, connected to the four-way valve servo and the motherboard. The motherboard is driven by the 12 VDC, as well as it provides down-converted power of 5 V and 3.3 V to other units.

Device	Voltage (V)	Max current (A)	Max Power(W)
Pump	24	2.75	66
Four-way valve servo	12	2	24
Nozzle servo	12	1.1	13.2
Motherboard	12	0.1	1.2
Flow meters	12	0.1	1.2
Pressure sensor	12	0.2	2.4
Temperature sensors	5	0.05	0.25
Thermal bus	5	0.15	0.75
LCD display	5	0.05	0.25

Table 5.7: Power consumption [1].

Power converter

The converter previously used was a Datel UCP-12/5-D24 DC/DC, Figure 5.12. This device is potted, in this case enclosed in a rubber like material, and occasionally malfunctioned during pressure tests. The reason for this was believed to be due to the potting, trapping air inside the enclosure and then damaging the circuits in the board when exposed to increased pressure. A power converter from SynQor, PQ24120QGA08 Power Qor, was acquired. This DC/DC converter uses MOSFET technology instead of the Schottky diode based technology used in most other common converters. By using MOSFETs the efficiency is higher and the heat dissipation much lower, making any heat transporting potting or metal plates unnecessary. There are also no aluminum electrolytic capacitors, which makes this circuit board more likely to withstand the pressure.

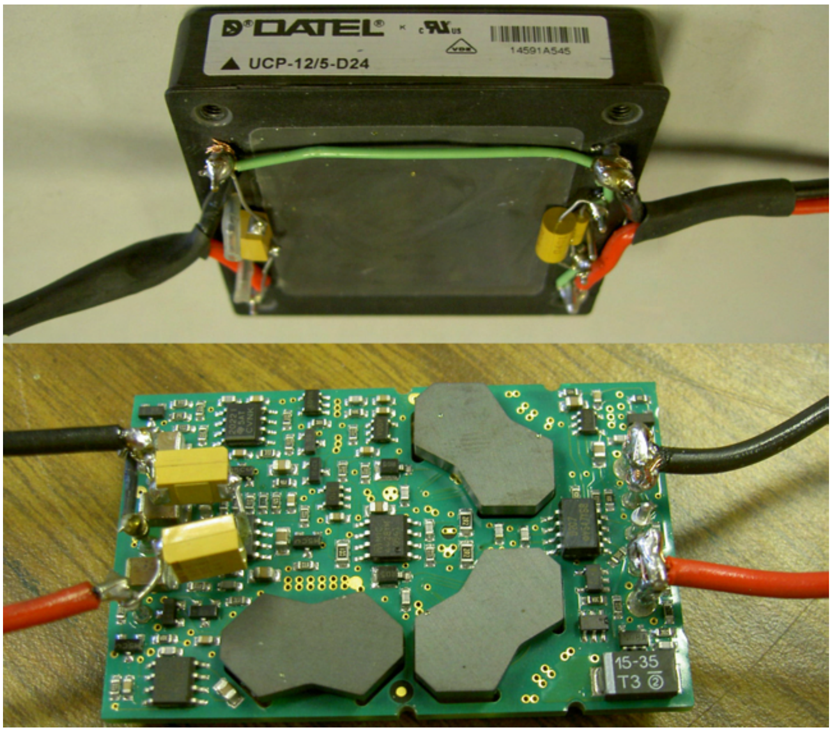

Figure 5.12: The old 24/12 VDC power converter in the top of the image, with the new one beneath.

Because of all the high-power inductive loads in the system, noise will be introduced in the wires. To solve this problem capacitors are attached between the power and return wires in order to filter out the noise.

Solid-state relays

The OWL2pe microprocessor on the motherboard is used to control the actuators and to run operations. The OWL2pe is able to source a maximum of 5 V and 25 mA to units connected to one of its ports. In order to control the flow of power of higher voltage units, such as the pump and the servo motor,

solid state relays (SSR) are used as digital switches. By sending an enable or disable command from the microprocessor through a power logic controller to the SSR, the higher power line is switched between connected and disconnected. The SSRs used there are the CMX60D10 from Crydom and contain no moving parts, thus are more reliable than other mechanical counterparts. These SSRs are capable of handling power loads of up to 60 V and 6 A, enough for the HVB system.

Power logic controller

In order for the microprocessor to control the array of SSR's without using too many I/O ports a power logic controller, TRIC6B595 serial to parallel converter, is used. This unit takes in a serial data stream from the microprocessor and converts it to a parallel, each bit going to a different output on the converter and thus controlling the status of a different SSR or other unit, Table 5.8. If a high voltage is received by the SSR it turns the connection on, and if it receives a low voltage it turns the connection off. The default statuses of the SSRs are in a disconnected state.

Port	Connection
0	Unused
1	Depth sensor
2	Unused
3	Flow Meters
4	Four-way Valve Servo
5	Nozzle actuator
6	Yellow status LED
7	Green status LED

Table 5.8: Power logic controller port assignment.

5.3 Actuators

In order for anything to happen in a system, there needs to be mechanisms to move and affect parts in a system, so-called actuators. The actuators are like the muscles in a body. They make things move and interact with the environment. The actuators for the HVB system are the nozzle motor, which opens and closes the intake, the four-way valve, which directs the flow through different filter assemblies, and the pump, which moves the water through the system.

5.3.1 Nozzle

The nozzle unit can be seen in Figure 5.13. In order to avoid contamination before and after sampling, the inlet on the nozzle needs to open at the right moment and provide a sealed closure when not sampling. The nozzle is made of aluminum and PVC plastic. PVC is a common material used in constructions and will serve as the main material for the nozzle housing.

The unit consists of two tubes, one inside the other. At the intake end of the tubes, holes are drilled through both of them. By moving the inner tube back and forth the holes can be made to line up, opening or closing the nozzle. The outer tube is fixed to the casing and the inner tube to the actuator inside. O-rings are used as a seal between the two tubes.

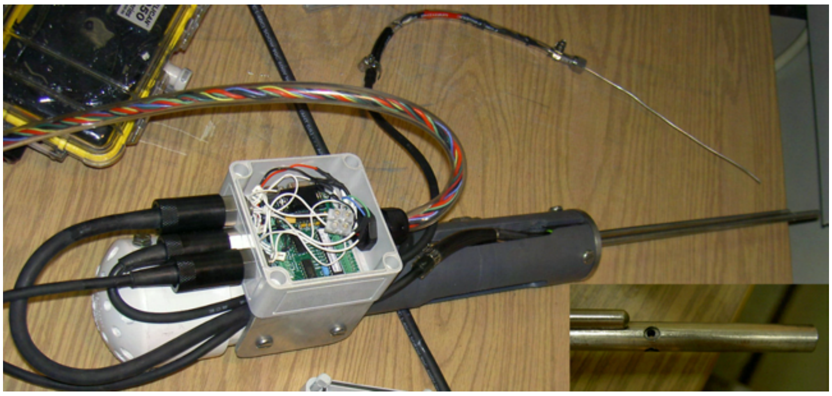

Figure 5.13: The nozzle under construction with a close up of the tip.

Nozzle servo

An early design of the nozzle used the same type of servo motor, SSPS-105, as for the four-way valve. Opposite to the four-way valve servo, which is situated inside the electronics box, the nozzle servo was exposed to the ambient environment. It was found that the casing of the servo motor could not handle the large differential pressure and had to be filled with Fluorinert. This still proved too tough, and the motor failed during testing.

A specialized linear actuator for underwater conditions from Ultra Motion LLC was used in the nozzle instead. This actuator uses a bipolar motor, HT17-075, instead of the unipolar motor of the earlier servo motor. The advantage with a bipolar motor is that the wiring of the motor is simpler, but this instead results in the need of more complicated control of the motor.

Servo control

A circuit, BiStep06 from Parallax, is used to control the nozzle servo motor. The controller is driven by the OWL2pe through a serial connection, using two wires, one for input and one for output. There is also a potentiometer built into the nozzle actuator to give feedback of the position of the nozzle, through a single wire. This information is used in the controlling software to determine the position of the nozzle during opening and closing operations.

Wires

The nozzle unit is connected to the main HVB body through a flexible hose, along which eight wires are run between the nozzle and the main HVB body. In addition to the three wires used by the servo, there is also one ground and two

power lines, 12 V for the motor and logic circuits and 5 V for the potentiometer. The two temperature sensors use two wires each, one ground and one signal. All grounds are connected to a common ground wire in the nozzle electronics box. The wires are then run along the nozzle hose in a tube, which is filled with Fluorinert liquid, to the main body of the HVB.

5.3.2 Four-way valve servo

The four-way valve is operated by a servo, a SSPS-105 from Tonegawa Seiko, connected to it inside the electronics box, Figure 5.14. The casing of the servo was removed due to its poor ability of resisting differential pressure and the risk of trapping air inside it. The servo is powered by 12 VDC from a solid-state relay, which, in turn, is controlled by an output port on the OWL2pe.

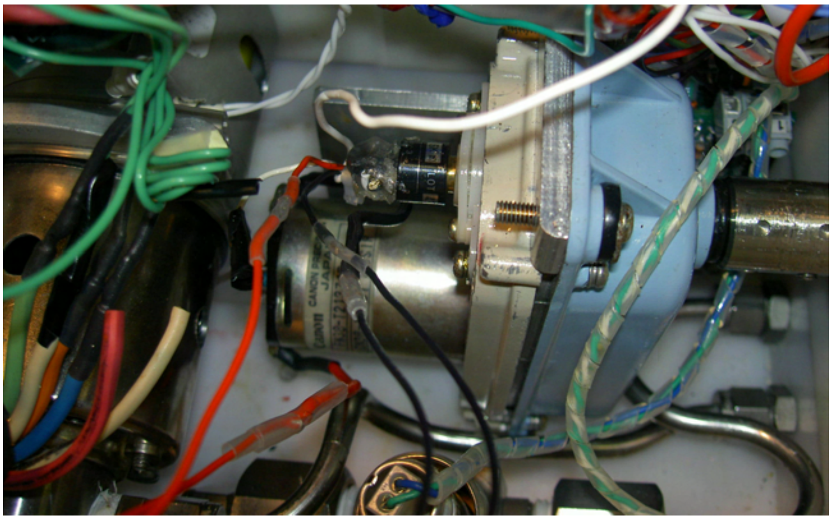

Figure 5.14: The four-way valve servo and the shaft connecting to the four-way valve to the right.

Servo control

Initially the servo used its own control electronics board, and the OWL2pe operated the servo by sending pulse-width modulation, PWM, signals to it. During earlier pressure tests there were problems operating the servo due to the pressures effect on the control electronics. Several electrolytic capacitors had to be replaced with the more pressure-tolerant variants, but there were still problems in running the servo motors. The result was that the control electronics board for the servo was taken out of the system, and that the servo instead was controlled directly by the microcontroller, switching its power on and off through one of the solid-state relays.

Potentiometer feedback

The position of the servo, and thus also the position of the four-way valve, is measured by a rotational potentiometer connected to the servo shaft inside

the electronics box. The software checks the readings from this potentiometer while turning the servo to stop at values representing the four different valves. The potentiometer is constructed with a 4:1 turn ratio, meaning that for every complete turn of the servo motor the potentiometer makes one quarter of a turn. This results in four different values on the potentiometer for each position of the servo motor to align with a valve. Some of these potentiometer readings could not be used, since the readings were unreliable in the area where the potentiometer completes a turn and starts over from the beginning. The four-way valve system is also prone to misalignment over time due to the continuous potentiometer, and calibrations are needed to ensure right positioning of the valves.

5.3.3 Pump

The pump that pulls the vent water through the nozzle, all the pipes and the filters, is placed in the electronics box. It is connected to the pipes after the manifold and is thus a common unit for the bypass as well as for the filter lines. It is a peristaltic pump, which means that it uses mechanically produced waves on a flexible tube to pump the fluid along its way. This is a technique used in the gastrointestinal tract and other biological systems. The unit consists of two parts, a DC motor and a pump head, magnetically coupled together. The pump is powered with 24 VDC directly from the power supply via a solid-state relay, which, in turn, is controlled by a port on the OWL2pe. The casing of this unit was kept on, so a number of holes have been driller through to let Fluorinert inside.

The pump is placed at the end of the flow system, pulling water through the pipes, instead of pushing it as if it had been connected in the beginning of the pipe system. The advantage of this is for the pristinity of the system and that the pump will not be exposed to the higher temperatures, as it would have been if situated in the beginning of the pipe system. Since the pump is placed after the filters, the water going through the pump has already been sampled, and there is no need for the pump to be cleaned and sterilized between each sampling. The downside of placing the pump in the end opposed to the beginning is that it is harder for the pump to pull than to push the water through the pipes. Due to the high resistance from the small pipes and all the fine-pored filters, the pump now has to work hard. This creates problems with a too small flow rate and decoupling of the motor, which means that the motor loses its magnetic connection to the pump head. It was measured, however, that the flow rate would be sufficient enough to reach the requirements of the system stated in Table 4.1.

To have the system primed with water from the start will also lower the effort of the pump to initially pull the water through an empty system. Having the pipes and filters filled with liquid is also a critical need when the system descends deep under the ocean surface, where any trapped air could implode the system due to the pressure difference.

5.4 Sensors

The system needs to sense its environment in order to interact with it correctly. This is done through different kinds of sensors, which is analog to the eyes and the touching sense of a human. The HVB has a number of sensors in order to monitor the system and its environment. Flow sensors are used to measure the flow through the system, to see that the pump is working and to calculate how much water is pulled through. The two different temperature sensors keep track of the sampling conditions, the temperature of the water being sampled and the temperature of the water at different parts of the system. A pressure sensor is used to read the ambient water pressure in order to determine the depth of the system.

5.4.1 Pressure

The pressure sensor is an SEB-50 from Seabird Electronics. This sensor is mounted on the metal-plate frame, to the right of the electronics box. It is a complete sensor system and rated to a depth of 6500 meters. The sensor use serial communication to send its data to the OWL2pe. These data are in ASCII format and are converted to a depth value by the OWL2pe.

5.4.2 Flow rate

To keep track of the flow through the system two FTB-9501 flow turbine meters from Omega Engineering are used, Figure 5.15. One of these is mounted along the piping inside the electronics box, reading the flow through a pipe common to the bypass line and the filter line. The other flow meter sits on the back side of the metal plate frame, connected only in the bypass line. The flow sensor connected in the back of the bypass line is used during the development phase and will not be included in the final system. The signal output from the flow sensor is connected through two wires to the FLSC-AMP amplifier mounted inside the electronics box, before it is received by the OWL2pe.

A problem with the flow sensor is that it is sensitive to the corrosive effects of the sea water passing through the system. The sensor uses a pelton wheel-like rotor, which is turned by the liquid flowing by. The rotor is connected to the sensor unit using small ball bearings. The frequency of the rotation is picked up by a coil, and is then translated to a flow rate. The mechanical part of the system was found to corrode by the saline water and the ball bearings were easily destroyed. It is therefore needed to flush the flow sensors with fresh water after a deployment to mitigate the corrosive effects of the saline water. The filters are important for the operation of the flow meters, since these sensors are actually only intended for clean fluids, and this is why the flow meters need to be placed after the filter lines as well.

5.4.3 Temperature

There are two different types of temperature sensors used in the HVB system, one is used for the high-temperature readings in the beginning of the pipe system and the other for the multiple readings by the filter arrays.

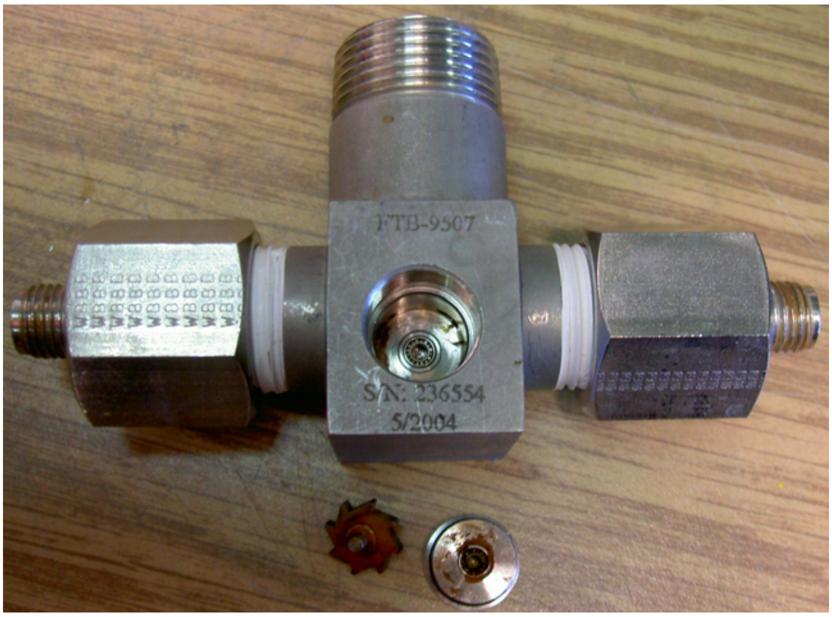

Figure 5.15: A flow meter, with its internal parts heavily affected by corrosion.

Nozzle sensors

One type is the thermocouple probes form Omega Engineering Inc. There are three of these high-temperature probes, and they are all placed in the beginning of the pipe system where the temperature will be highest. One is placed at the tip of the nozzle, HTTC36-K-316G-6, to measure the temperature in the vent plume. The other two, TJC36-CASS-040G-6, are placed further down the line, one at the end of the nozzle and the other just before the water enters the four-way valve. The water entering the nozzle can be as hot as up to 400 °C but will then progressively cool down as it flows through the pipe system. These high-temperature sensors are rated only to 230 °C and will have to be replaced with sensors rated for even higher temperatures in the final system. Two wires are used for each of the thermometers, signal and ground. In order to be able to decode the analog data, the signal lines are connected to the 12 bit ADC which provides temperature resolution of 0.25 °C.

Filter array sensors

For the filter part of the system another type of temperature sensors are used, DS 1822 from Maxim Semiconductor. These sensors are less temperature and water tolerant than the ones used in the nozzle, taking measurements in the region −55 °C to 125 °C. The advantage with these sensors is that they use the one-wire bus protocol. This bus uses three wires, power, ground and signal, and can even be configured to use only two, a shared power/signal and a ground wire. Several devices can be attached to this bus, each independently addressable by a unique address. This makes it possible to have a large array of these small temperature sensors along the filters, monitoring all the different temperatures, with a minimum number of wires.

5.5 Communication

No measurement system or instrument is any good if you cannot view the results. The main purpose of the HVB system is to take pristine samples for further studies in a well-equipped laboratory. However, the HVB system needs also some supervision and data logging abilities during its operation to ensure that the samples are taken at the right place and at the right moment. It will also need to know at what conditions the samples have been collected when they are to be analyzed in the laboratory. To do this, several different sensors logg the external and internal environment of the system. This data can be viewed in real time or at a later time when the stored data are read from the onboard memory.

The communication with the system is done through RS-232, a normal serial cable that is a widely used standard for transmitting serial data. During the development phase the system is connected to the ground station, a laptop computer seen in Figure 5.16, through a 150 foot long tether. When the system is to be deployed at a real hydrothermal vent, the HVB will be connected to the submersible vehicle. The control station will then be placed in the submersible, or in the research ship at the surface. In the latter case the submersibles communication lines with the research vessel will also be used to communicate with the HVB. With the RS-232 protocol for communication, the HVB system can be used by most terminal software for monitoring and control.

Figure 5.16: The control station on the ship during the Iceland test. The operator had visual contact of the nozzle and divers through the LCD, while supervising the operation of the HVB on the laptop.

5.5.1 Commanding the system

The keyboard is used to command the HVB by pressing different keys and thereby sending different ASCII signs, Table 5.9. Upon reception, the command is interpreted by the operating software and acted on appropriately. All commands are single signs except for the text message to the LCD, which is a string of up to 32 characters. There is no buffer to store the received data, so the commands can only be received by the system when the software is listening on the serial input. If the command is sent while the system is busy doing something else, such as operating actuators or sending data, the command will not be taken. With LabVIEW as the operating software on the ground station, a software buffer can be implemented that will hold the command until the system is ready to listen.

Command	Function
A-I	Set the update rate, 1-9 s
a	Turn off pump
b	Turn on pump
c	Open nozzle
d	Close nozzle
e	Send data to station immediately
0-3	Positions for four-way valve
	0 = bypass, 1-3 = filter lines
m	Send text message to LCD
M	Clear text message from LCD
p	Stop data logging
r	Resume data logging
s	Start data logging
T	Clear flash memory
U	Upload data from flash memory

Table 5.9: Software operation commands.

5.5.2 LED

There are four LEDs on the motherboard that are used as status indicators of the system. These LEDs are used for debugging and testing, but are also used as visual status indicator of the HVB operation during deployment for an assisting diver, Table 5.10.

LED	Color	Connection
D1	Red	System power
D2	Red	Unused
D3	Yellow	Four-way valve motor
D4	Green	Nozzle motor

Table 5.10: LED indicators.

D1 is connected to a 5 V port on the OWL2pe and is thus used as an on/off power indicator. The two motor LEDs, D3 and D4, are turned on and off with

the power to the motors through the power logic controller. D2 is currently not in use.

5.5.3 LCD

The LCD has two display lines of 16 characters each, but this will be replaced with a four-line 20 character version. It is connected to the motherboard through a power, ground and signal wire for serial communication. The LCD is used mainly for the testing phase of the HVB and will not be part of the final system, as there is no need for its operation then. During testing, the LCD displays data and operational commands, which are sent to the HVB. The control center, the computer above the surface from where the HVB is controlled, can send messages down to a diver through the LCD. Though this is only a one-way communication, it can still be very valuable and save time compared to the diver resurfacing each time to get new instructions and updated on the operation. The cursor also helps to indicate the status of the system. A blinking block-cursor is used when the instrument is taking measurements. When the system is listening for commands from the control station the cursor is a steady underscore, and if there is no cursor the system is busy with processing or controlling the actuators.

5.5.4 Debug window

The blue PBASIC debug window gives the control station an interface with the system to receive data and send commands. This is done through the RS-232 interface in a half duplex communication over the transmit line and the receive line. Since the OWL2pe does not have a receive buffer for commands, the system can receive commands only from the control center through the debug window when the program is listening. Either one has to continuously strike the key for it to be caught by the software at the right time, or one can use the LabVIEW interface described below, which provides a software input buffer.

The format by which the data is displayed in the debug window can be of two forms. One is as a long string with all the data, separated by comma signs. These data printouts create a list in the debug window of data sample sets, and one can scroll through the list to see trends and changes in the data. Another format is a more user-friendly labeled window. The labels tell the viewer what the different values are, but only displays the latest sample measurement received. In this mode one is thus not able to follow any trends in the received data.

5.5.5 LabVIEW window

The LabVIEW provides a graphical user interface (GUI) to the control and operation of the HVB, Figure 5.18. The so-called front panel of the GUI is divided into eight different pages. The first page is the main, showing the measurement data, and has the controls to command the system. There is a Systems Schematics page that shows the measurement data as a flow diagram over the pipe and filter assembly, with the data from the different sensors placed along the way. The other pages show the temperature, depth, flow and power in a chart over time. The Settings page allows for changing some variables of the graphical display in the LabVIEW. The last page simply displays the raw data

received from the system. The LabVIEW interface provides a command buffer. Right after that LabVIEW has received data from the HVB any command in the buffer is sent.

5.5.6 Onboard memory

The data can be stored in the onboard memory and then read back later. This feature is enabled by a command from the control terminal and can be activated simultaneously with the other forms of outputting the data above.

5.6 Software

The software used with the HVB system is the PBASIC language to program and control the OWL2pe microcontroller. Additional software used is LabVIEW, which provides a graphical user interface (GUI) for the operator at the control station.

5.6.1 PBASIC

The HVB control program is written in PBASIC which is an abbreviation of Parallax Beginner's All-purpose Symbolic Instruction Code. This program is designed from the BASIC language, specially adapted for being used with the Parallax Stamp family microcontrollers [8]. PBASIC is a simple but yet powerful language to control these processors.

The BASIC Stamp must be able to distinguish between operational commands sent to the system and programming commands. In order to load a program into the BASIC Stamp, a programming protocol on the serial lines is followed. It starts by sending a signal, a 2 ms high voltage pulse, on the DTR line from the computer, which is connected to the ATN line on the BASIC Stamp unit. This signals the BASIC Stamp to reset. For programming, the TX line is also set high for additionally 36–38 ms, which indicates to the BASIC Stamp that it is to be programmed. To avoid any accidental reprogramming, any time outside this time interval will not be accepted as a reprogramming command.

Of the non-volatile memory 16 kB are dedicated for program storage, divided up to eight 2 kB slots. The HVB operating software written in PBASIC is divided up as well in different parts and stored in these 2 kB memory slots. The grouping of the programs is according to functionality and program operation, such as all actuator operations are collected in one slot and measurements are collected in another. The HVB control software is comprised of the programs shown in Table 5.11 and their relation during the software operation can be seen in Figure 5.17.

Each program in the different program slots must have the same definitions of variables in the beginning as an identical header. This is to ensure that when switching between the running programs no memory corruptions will occur when the new program defines its variables in the 26 bytes RAM. BASIC Stamp allocates space to the variables, starting with the largest variables, such as Words, followed by Bytes, Nibs and finally the Bits. Within these size groups the Stamp goes by the order in which the variables are defined. If the lists of

Slot	Program	Memory usage of the 2kB
0	hvsInit.bpe	≈0 %
1	hvsMain.bpe	60 %
2	hvsActuate.bpe	39 %
3	hvsMeasure.bpe	27 %
4	hvsOutput.bpe	15 %
5	hvsLogging.bpe	79 %
6	unused	0 %
7	unused	0 %

Table 5.11: Program memory allocation.

the variable declarations are not identical between the different programs, the data in the variables will be corrupted, and the programs will most likely crash.

When the PBASIC code has been written and run, the code is tokenized and stored in the OLW2pe. The BASIC Stamp then uses an interpreter to read and run the code. Tokenization of the source code is the procedure of transforming the symbols and commands to another format in order to save room, for instance a number. This is a lossless compression system used in most BASIC interpreters. Another way for programming languages, other than to tokenize the code, is to create an executable code.

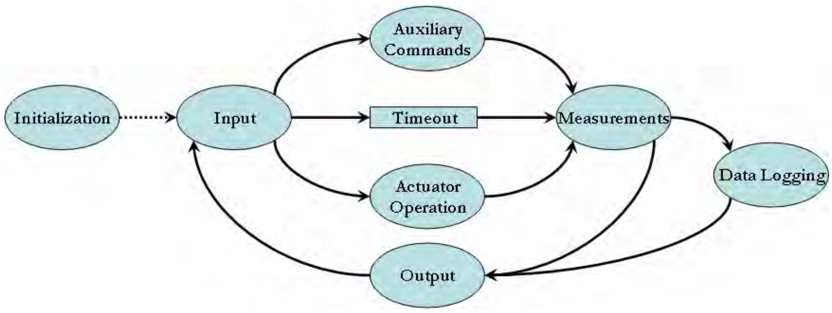

Figure 5.17: Software operation schematics.

hvsInit.bpe

hvsInit.bpe is stored in program slot 0. When the system is powered on, the program stored in this slot is the one to get executed first by default. This program initializes the system and all of its components.

hvsMain.bpe

After the system has been initialized the hvsInit.bpe calls on the hvsMain.bpe code stored in the next memory slot. This is the main program for the HVB operations. This program keeps track of the system and calls the other different subprograms in sequence to perform certain tasks. This program also has the input routine for commands sent from the control station. If a command is received from the control stations keyboard, then the main program calls on the

appropriate program or subroutine to act on it. The system will be waiting to receive a command for a certain time before moving on with other procedures.

If a so-called auxiliary command is sent, the program calls on a subroutine to set the update rate, enabling or disabling data logging, sending a text message to the LCD display or accessing the flash memory. The data logging is disabled at startup by default. The update rate sets the time the program waits for an input before timing out and moving on. This decides how long every program cycle takes and thus the time in between taking new data measurements.

hvsActuate.bpe

In case of an actuator command the program will send the commands to open or close the nozzle, turn the four-way valve to the requested position or to turn the pump on or off.

hvsMeasure.bpe

Whether the system has just acted upon an auxiliary command, actuator command or if the command timed out, the system continues to take in measurements by running hvsMeasure.bpe. This program receives data from the system and its sensors, and stores these temporarily in the memory. The data include a time stamp from the real time clock along with values of sensors and system status, such as the battery voltage, four-valve position, pressure reading, flow rate and temperatures.

hvsLogging.bpe

In case the data logging feature was activated by the auxiliary command, this is the next program gets called. The data just measured are stored in the flash memory, one block of measurements after another, until the data logging feature is disabled. The stored data can later be retrieved from the flash memory. The flash memory is a non-volatile memory, so the data will be stored here even when the power is cut, until the memory is cleared or written over.

hvsOutput.bpe

The data just gathered, whether the data logging feature was enabled or not, will next be displayed on the debug window on the control station. From this data feature, commands and operations can be planned. When this is done the program will go back to the beginning of the main loop and start the cycle over again.

5.6.2 LabVIEW

LabVIEW is a graphical programming language and is used to give the operation and data retrieval from the instrument a more user-friendly interface, Figure 5.18. The programs created are called virtual instruments (VIs) because of the imitation of actual physical instruments [7].

A set of tools and objects is used to create the interface, compared to the PBASIC language, which is text-based. LabVIEW lets the data flow decide

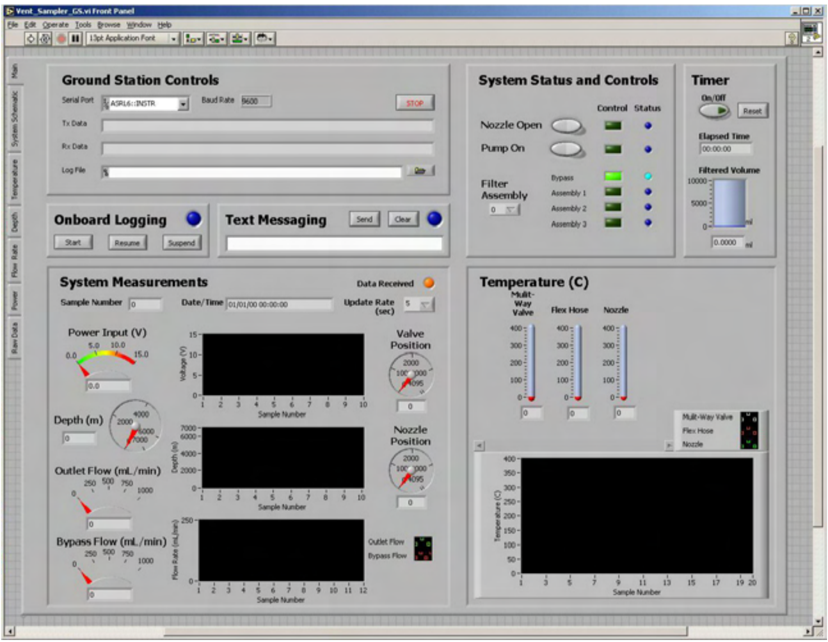

Figure 5.18: The LabVIEW environment.

the execution of the program, so-called data flow programming, instead of the text-based and instruction-determined program execution used by PBASIC.

The LabVIEW programming environment comprises two main windows, the Front Panel and the Block Diagram. The Front Panel is the user interface and can be built up with different controls and indicators. Controls are input devices used to control the program and include knobs, push buttons, dials and more. The indicators are output or display units such as graphs, LEDs and numerical displays. The Block Diagram contains the code and the relation between all of the devices in the Front Panel, looking much like a flow chart.

Chapter 6

System testing

Developing the HVB system has been an iterative process, going from building and modifying the system, to tests and analysis, and then back to building and modifications again. Simpler and smaller tests can be performed in the laboratory, while others require the system to be taken to the ocean or to a pressure chamber. During the tests one can see how the system will act in real life, and problems one never thought off might arise. "Test as you fly, fly as you test" is a motto that states that you should try to test the system in an environment as close as possible to the one it is intended for. Then when you are actually going to use the system, do it in an environment as close as possible to the one in which the system has been tested.

6.1 Lab testing

The testing of the hostile environment where the HVB is designed to operate is very limited in the lab. For the pipe and filter system there is a pressurized water tank from where water can be run through the system, Figure 6.1. This is a good system to test for leakage and to see how the filters and pump will perform under a higher pressure. It was actually found that the pump performed better with a closed pressurized pipe system with pressure of at least 3 bars, than with the ambient atmospheric pressure of 1 bar.

One major issue with the system has been the flow rate through the filter array. At first the pump was not able to pump the water through the system. If the system has a too low flow rate, it will take too long to sample a certain amount of water. If the system has a too high flow rate, then any biology captured in the filters could be destroyed. Throughout the work with the HVB the flow rate was too low and even non-existent. The major flaw in the flow rate was found to be the 15 μm filter. For some reason this filter gave the highest flow resistance and it was excluded in the latter test in Iceland. The system now has a flow rate of 60–80 as the readout from the flow sensors, which corresponds to 0.6–0.7 liters per minute. This flow is believed to be just enough for the operation of the system and not too high to damage any microbes trapped in the filter.

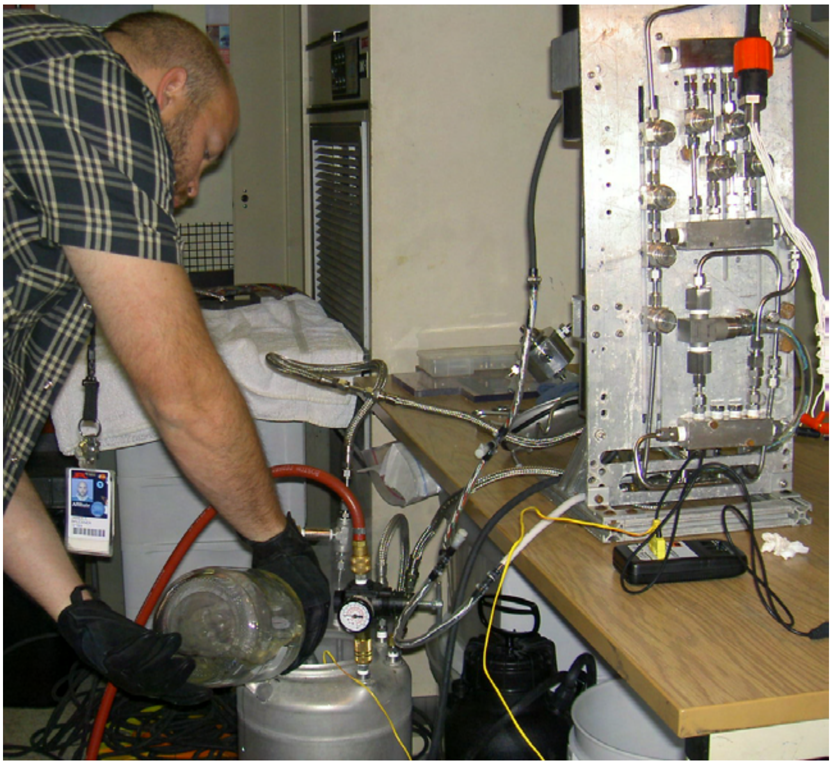

Figure 6.1: Testing the HVB in the lab using an external pressure tank.

Heated water was run through the system to test how various components and sensors react to the increased temperature. The temperature has, however, been limited, naturally, to just under the boiling point of water. Tests caused no problems, but the system needs to be tested under even higher temperatures.

6.2 Scripps Institution of Oceanography

Scripps Institution of Oceanography in San Diego, California, was founded in 1903 and named after Ellen Browning Scripps and E. W. Scripps. In the beginning Scripps was an independent biological research laboratory, but in 1912 it became a part of the University of California. The research is undertaken in the fields of physical, chemical, biological and geophysical studies of the oceans. The institution is staffed with about 1300 people, of which about 200 are graduate students. The annual expenditure is over $140 million.

The HVB was taken to Scripps on December 16, 2005, to verify that the system will be able to withstand and operate under the extreme pressures experienced at the bottom of the oceans. Some of the hydrothermal vents are situated at depths down to 6500 meters. At these depths the pressure is 651 atm or about 9600 PSI. At Scripps the HVB was tested in the Ocean Atmosphere Research (OAR) facility in a pressure chamber which put the system up to 10,200 PSI (703.3 bar), equaling a depth of 6941 meters below the ocean surface.

Before the system was put to the test, it was primed with water so that

no trapped air would cause any implosions. This was done by running water through all of the pipes and filters. In a real scenario this would be sterilized water to avoid any biological contamination to end up in the filters. After the system had been primed it was put in the pressure chamber, a massive vertical steel cylinder with a removable lid, Figure 6.2. The HVB was sealed inside this cylinder, which was then filled with water. Water is a very incompressible liquid and just a little extra water needs to be pressed into the cylinder in order to raise the pressure inside substantially. The pressure was stepped up until the level of 10,200 PSI was reached, which took about half an hour. During this time the system was turned on, and the pump was run for different pressures for both the bypass and filter assembly. During this test the system sent back data to the control station with the conditions inside the cylinder, such as temperature, flow rate and simulated depth.

Figure 6.2: Pressure testing at the SIO OAR facility.

The pressure test at Scripps Institution of Oceanography was successful. At earlier similar tests the system had failed due to the high pressure. Units such as the LCD display and the 24 V to 12 V power converter malfunctioned. The probable cause to this is trapped air within the units, which caused implosion and destroyed the units. Outside the box an earlier version of the 0.2 μm filter could not withstand the pressure and also imploded.

During this pressure test none of the components seemed to fail, but later back at the lab it was found that the LCD and the power converter malfunctioned. The LCD is not a critical component, as it will not be included in the deep dives with the system, but the power converter must work. Another power converter was acquired after this and it uses no padding or aluminum electrolytic capacitances. This component will be implemented in the system for the next pressure test.

There was also a malfunction with the flow through the system, where the bypass flow-meter showed a flow even though only the filter line was enabled. This error was noted at 3000 PSI, and the flow displayed increased with the pressure. This corresponds well to the pressure limit of the four-way valve set

by the manufacturer, at 3000 PSI. A valve with a higher resistance might be needed for the deep-sea version. The check valves probably also need to be changed, since they are rated only for 6000 PSI.

6.3 Off-pier ocean test

The off-pier ocean test was performed on January 25 by the coast of Los Angeles, at Hermosa Beach, Figure 6.3. The purpose was to see how the system would cope with the ocean water and to test the deployment and operation of the system using the new 150 foot tether. In addition, the new 0.2 μm filter had just been attached and incorporated into the system.

Figure 6.3: Deploying the system off the Hermosa Pier.

At Hermosa Beach the HVB was brought out to the end of the pier. In-the-field system set-up and deployment procedures were tested. The system

was run with two car batteries connected in series as power source, providing a voltage of 24 VDC. The HVB was then deployed into the water from the pier, hanging in the tether by a load-relieving Yale grip. Deploying the HVB this way tested and verified the strength of the tether. Well in the water the system was submersed down to the maximum depth of 9 meters where the bottom was, as determined by the HVB depth sensor.

The operation could be observed through the data received by the computer, in the debug window, as well as in LabView. There was also a camera attached to the structure showing a view of the LCD display and the four-way rotation shaft in the electronics box. This way the system could also be visually observed during the system testing. The test was successful, except for some complications with the flow rate through the filter assembly. This was, however, expected since the ambient pressure when the system was fully submerged was not high enough, only barely reaching 2 atm. The pump had in earlier tests in the lab been found to start operate well enough at above 3 atm pressure inside the pipes. Modifications to the system to get the flow rate up will have to be made if the system is to function at these shallower depths.

6.4 Iceland test at a real hydrothermal vent

On March 27, 2006, the HVB was taken to Akureyri on Iceland, where it was tested at a real hydrothermal vent in the fjord by the town, called Eyjafjördur, Figure 6.4. The HVB system had been here for tests the year before, but then the system did not function as hoped, and no pristine samples were collected. The system had now been modified in many aspects after the first Iceland test, such as being outfitted with new filters and a new nozzle.

Figure 6.4: Akureyri and the Eyjafjördur with the known vent sites marked, Matthias Bjarnason.

The test in Iceland was made in collaboration with the local university, Háskólinn á Akureyri. An hour-long boat-ride out in the fjord from Akureyri there are two areas of underwater hydrothermal activity. These are named

Ystuvíkurstrýturnar, discovered in 1990, and Arnarnesstrýturnar, discovered in 2004. These vent systems belong to the shallow vent-class, a designation given to underwater hydrothermal vents found at depths less than 1 km.

The hydrothermal vents in the Eyjafjördur spew out mostly fresh water, containing only about 1 % sea water. These vents, which are within scuba diving reach, are different from the ones found much deeper. The fauna in their surroundings is not that dependent on the vents, and the chimney structure is made up of magnesium silicate clay instead of the anhydrite at the deep-sea vents. However, being within reach of scuba divers to assist, these shallow vents provide a very good test environment for the later sampling at the deep-sea hydrothermal vents. It is also very interesting to see what can be acquired from these vents biology.

The Ystuvíkurstrýturnar consists of three major hydrothermal vent pillars situated at a depth of 65 meters. The tallest of these reaches 50 meters above the bottom, and thus the top is situated some 15 meters below the ocean surface. The temperature of the vent water has been measured to be 72 °C. These vents are older than the Arnarnesstrýturnar, where the tests were performed, and the Ystuvíkurstrýturnar are now situated in a protected area.

The Arnarnesstrýturnar consists of several small vents, situated along a 750 meter long stretch, at a depth of 25-40 meter. As with the Ystuvíkurstrýturner, the chimney structures reaches to about 15 meter below the surface, which seems to be the depth to which the chimney structures can reach. This is believed to be an effect of the waves in the fjord, restricting chimney growth.

The particular site at where the test was performed was a group of vents some 16 meters below the surface, having vent plumes reaching a temperature of 78 °C. Unfortunately, the temperature-probes on the nozzle malfunctioned, showing no or unreliable data. However, a temperature of 77.1 °C was measured with an external temperature probe inserted by a diver. The cause of the temperature sensor malfunction is thought to be the long wires from the sensors in the nozzle, leading to the main unit and the sensor electronics there. When actuating the nozzle servo the temperature readings increase as well. Introduced noise with the other lines and a possible potential drop over the temperature wires could be the reason for their malfunction.

A deployment and operations protocol had been established before, so that both the operators by the control station and the divers would know the procedure, in case of a communication breakdown. The HVB system was deployed from a boat above the site, Figure 6.5. The system had previously been cleaned with a hydro peroxide solution through all the pipes and filters. Then the system had been flushed and primed with sterile water. When the system was submerged at the surface, it was run in bypass mode, to verify that the pump, flow sensors and temperature sensors were operational. The LCD for text messages and the camera was also checked to ensure that communication could be established with the divers through the tether connecting the control center with the HVB. Next, the two divers descended with the system to the bottom under the boat, 25 meters below the surface, and then transported the system to the location of the vent. The HVB system had been provided with a number of flotation devices to make it neutral buoyant in the water. This made it much easier for the divers to handle and transport the HVB. At the vent, the HVB was placed on the bottom next to it. The nozzle was inserted into the vent, after which the diver signaled to the control center to start the operation, Fig-

ure 6.6. First the HVB was run on bypass to flush the system before turning to the filter line and start the sampling. The system was then run for a period of time before the nozzle was closed and the HVB taken back to the surface. The 0.2 μm filter was then back-flushed to retrieve anything collected there. The last thing to do was to clean the system and prepare it for another deployment. The turn-around for this was about 30 minutes and this procedure could be done on the boat at the deployment site.

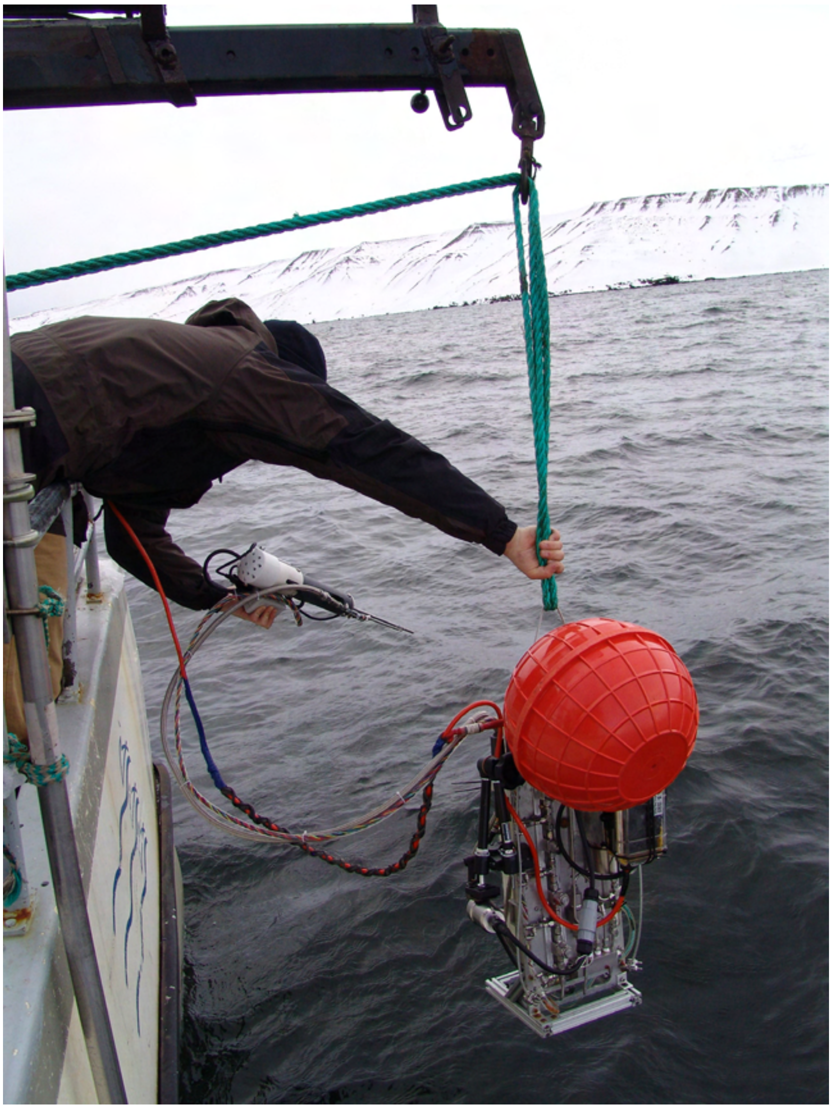

Figure 6.5: The HVB, with a buoy attached, being deployed off the boat above the vent site.

The weather conditions only allowed two days of testing. The first deployment gave the HVB a sampling time of 14 minutes. The operation of the HVB was restricted by the time the divers could spend at the vent, supervising the

Figure 6.6: Diver behind the vent with its warm water plume and the nozzle inserted for sampling.

system. During the first deployment, the camera malfunctioned and the nozzles open-and-close mechanism had problems operating correctly. The nozzle malfunctioning is thought to be due to the potentiometer, feeding back the position of the nozzle to the operating software, was affected by the colder temperature. Therefore the resistance changed a bit and the readings differed for the two stop positions. A mechanical switch might instead have to be used to control when the nozzle has reached its open and close positions.

The operation of the nozzle could, however, be solved by editing the software, and the sample could be taken according to the predetermined procedure. After this test the weather conditions did not allow any further attempts for several days. In the mean-time the system was trimmed and made ready for favorable change in the weather conditions.

This change came on the day before the departure from Akureyri, and the system was once again taken out to the vent site in the fjord. For this second deployment the HVB was taken down to the vent by a pair of divers, and then left there running for over one and a half hour. In the mean-time the divers ascended, and when the system had run for a while, another pair of divers was deployed to retrieve the system from the vent. This showed that the system can operate in the water by a vent for a longer period of time than required.

The two tests were successful. With a flow rate during both deployments measured to just over 0.6 liters per minutes and with a consistent flow during the whole deployment. About 9 and over 52 liters of water were thus filtered during the two deployments. The contents of the filters were put in containers and frozen, to later be analyzed for DNA in the Biotechnology and Planetary Protection Laboratory back at JPL. Some samples were also left to be analyzed at Háskólinn á Akureyri. The procedure for analyzing the samples can be found

in the next chapter.

6.5 Back to Scripps Institution of Oceanography

After the Iceland test the HVB went back to Scripps Institution of Oceanography on April 21 for another pressure test. The reason was that the unit had been equipped with some more components, especially the new nozzle and power converter, and it was necessary to see how these would withstand the pressure.

The nozzle operated at a pressure of 6000 PSI, where it ceased to actuate when commanded. Before this the nozzle opened and closed as it should, and when taken back to a pressure of 5500 PSI the nozzle operated as usual again. What the cause of this malfunction is, and why it happened between 5500 and 6000 PSI will have to be looked into further.

The old potted 24 V to 12 V power converter had been replaced with the new one, and this one proved to be functional to the required pressure of 10,000 PSI. This unit has now been mounted on the inside wall of the Delrin box, as part of the HVB system.

During this second pressure test there was no indication of any flow through the wrong line, as for the first test. In between the two pressure tests as Scripps the pipes and the fittings were tightened. The connections to the shaft of the four-way valve were also further sealed with Teflon tape. This prevented the leakage seen earlier and thus solved the problem.

Chapter 7

Bio-analysis of samples

The samples collected from the Arnarnesstrýturnar vents in Eyjafjördur on Iceland will be used to test the bio-assay procedures for the HVB project. During the sampling at the vent the water was flushed through the 0.2 μm filter, where the microbes of interest should have been trapped. In order to back-flush the filter, an external pump has to be attached to one end of the 0.2 μm filter. Some 500 ml of sterile water are then pushed backwards through the filter, releasing anything trapped in the filters, and flushing it out with the water through the other end. The three-way T-junction can be seen in Figure 7.1, at the bottom of the 0.2 μm filter, where the external pump is connected during back-flushing. This water is tapped onto sample bottles for storage until analyzed.

The collected sample was split in half, one half going to Háskólinn á Akureyri, where it is to be analyzed with culture-based methods. The other half was taken back to the Biotechnology and Planetary Protection Group at NASA JPL in Pasadena, where molecular-based methods will be used to analyze the collected samples. In the case of taking the samples back to NASA JPL the bottles with the fluid were kept frozen with dry ice, frozen CO_2. The three 90, 60 and 7 μm filters were also extracted from the HVB and will be analyzed by the Háskólinn á Akureyri to see whether any particles or biology could have been trapped there.

Molecular-based analysis

The procedure for the molecular-based analysis has been worked out by Bruckner at the Biotechnology and Planetary Protection Group, NASA JPL, Pasadena, USA. The molecular-based analysis consists of an initial nucleic acid extraction, which is followed by the use of the polymerase chain reaction, PCR, to examine the microbial diversity.

PCR is a technique used in molecular biology to replicate DNA strands without directly using any living organisms. Through PCR a small amount of DNA can be replicated many times, resulting in an exponential increase of the targeted DNA region. Such amplification of DNA is required for most modern molecular assays. In order to amplify the right segment of the DNA the right primers must be used. The primers, which themselves are short artificial DNA strands, selectively adhere to the DNA, flanking the region to be replicated. It is then between these endpoints created by the primers that the new DNA

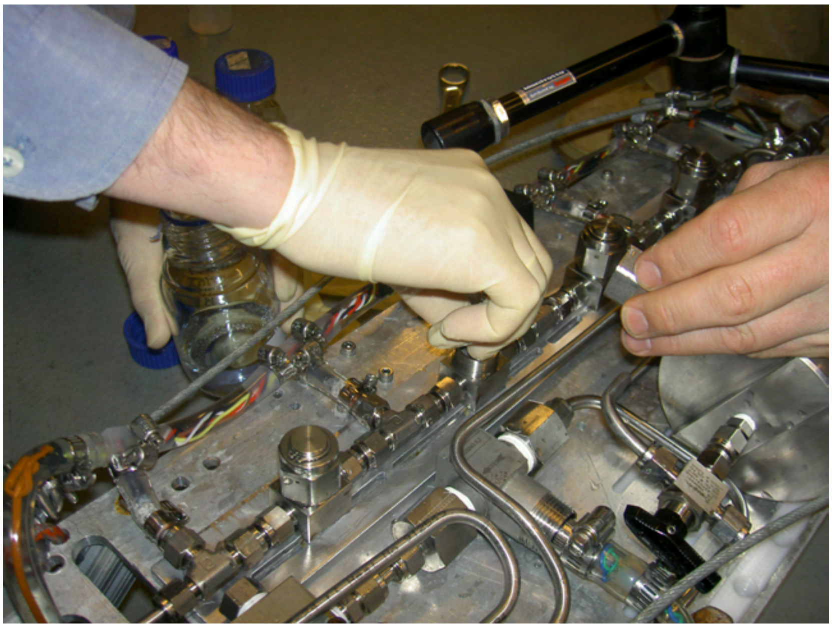

Figure 7.1: Filters being extracted after a sampling test from the HVB for analysis.

strand will be synthesized.

The primers used in this analysis are directed at the universal bacterial and Archaeal 16S rDNA. Used in parallel to this are also primers for targeting the chemosynthetic functional genes, *acl*B, *oor*A, *por*A, *cbb*L, *cbb*M, *mcr*A, *pmo*A and *dsr*AB.

In order to see the microbial diversity based on the universal primers, a molecular cloning technique will be used in combination with a computer-based phylogenic analysis. Through the amplification of the chemosynthetic functional genes the presence or absence of the targeted metabolic pathways within the hydrothermal vent community can be found.

Primers

The universal primers used are directed towards the two branches of prokaryotic life, bacteria and archaea. These primers should amplify a specific region of the DNA from nearly all organisms of the specific type. For example, the bacterial universal primers amplify the DNA from the bacteria only, in an exponential manner. Primers targeting chemosynthetic genes will selective amplify DNA from microbes potentially able to use the targeted metabolic pathways. The presence or absence of these genes in the hydrothermal vent environment will help define the microbial community (it should be noted that absence of a target does not necessarily rule out absence of specific metabolisms).

Molecular cloning

Through molecular cloning the natural properties of the bacteria are used in order to selectively pick out individual pieces of DNA from the PCR-amplified mass, as described earlier.

Bacteria contain small regions of DNA, so called plasmids. A plasmid is an extra-chromosomal ring of DNA capable of autonomous replication. These plasmids are separate from the bacteria's single circular chromosome and are mobile, which means that the plasmids can be transferred or picked up from the environment by bacteria. Only one specific plasmid can be picked up by an individual bacterial cell. Plasmids are also self-replicating, meaning they can make copies of themselves from within the bacterium. As bacteria reproduce asexually, making identical copies of themselves, the plasmids within the original cell will also be present in the daughter cells.

The procedure

By putting the PCR-amplified DNA with specific "opened" plasmids, each plasmid will pickup by a piece of DNA and reform (a process termed ligation). These ligated plasmids are added to specially conditioned bacterial cells, that readily pick up plasmids from the environment (a process referred to as tranformation). As each individual bacterial cell may pick up only one of the plasmids, the result is many bacterial cells containing one plasmid each, which themselves each contain one piece of the original PCR-amplified DNA.

With culture-based methods the individual bacteria colonies can then be isolated. A bacteria colony comes from one single cell that has undergone many rounds of asexual reproduction, making up 10^4–10^6 identical cells, each with a plasmid inside. The plasmids are isolated from an entire colony, and the ligated DNA within excised. The ligated DNA from all plasmids will be identical (hence the term molecular cloning) and will be representative of the DNA from a *single* hydrothermal vent organism. It is now possible to determine the relation of this bacterium to any other by its DNA sequence through phylogenetic analyses.

Chapter 8

Discussion and conclusions

Throughout the work with the HVB, the system has undergone many changes in both its hardware and software. It has evolved to a more complete system, but has yet some way to go before it will be able to reach all requirements set for the project.

The development of the HVB for the extreme conditions the system will face, using off-the-shelf components, is a challenging task. Each new component needs to undergo pressure tests before being integrated into the HVB system. After these tests several areas of needed improvement are usually found and will have to be considered.

The two K-type thermocouples in the nozzle give unreliable data. The cause of this might be the signals from these sensors that travel a long distance through wires before received and interpreted on the motherboard. Therefore it is thought that noise and interference are introduced along the way, which degrades the sensor performance. This problem will have to be looked into and solved, since the nozzle temperature is the most interesting scientific data measured when collecting samples from a hydrothermal vent water plume.

The digital thermometers attached to the filters on the back side of the HVB are influenced by the colder ambient sea water. The sensors show the same temperature as the ambient ocean water. It is desirable to monitor the vent water temperature as it progresses through the pipes, and therefore the digital temperatures need to be better coupled to the sampled vent water running through the filters and also isolated from the much colder ambient seawater so this will not influence the readings.

The flow meters have occasionally lost data during testing. It was found that the fine mechanics inside, especially the ball bearings, were heavily affected by corrosion, due to the saline seawater. To mitigate this problem the pipes need to be flushed with fresh water after saline water has been run through, and preferably also dried by pressurized air blown through. A more easily accessible configuration of the flow meter might also be necessary, in case in-the-field replacing will be needed.

The dual flow problem experienced at the first pressure test at SIO seems to be solved. There was flow registered through both the bypass line and the filter line. This dual flow first showed up at about 3000 PSI, and the differences between the flows through the two pipes decreased with the increasing pressure, showing no difference at all at full pressure. After tightening and securing the

connections, the second pressure test showed no indication of any dual flow.

The nozzle actuator had operation problems during the Icelandic test. The actuator performance seemed to be degraded by the colder temperature experienced during this test, and modifications had to be made in the software to fully open and close the nozzle. Why this happened needs to be investigated further, but it is thought that the potentiometer, which decides the position of the nozzle, is affected by the temperature. A solution to this would be to use switches instead, to determine when the nozzle is open and closed, but this, in turn, will result in the need of additional wires between the nozzle and the main HVB body. A software solution, taking into account the ambient temperature, would be a better solution to this nozzle problem.

The HVB system has, after the tests these last six months, showed that it is able to operate for over 1.5 hours, in an ocean environment and under higher pressures, while taking samples from a hydrothermal vent. The full system still needs to prove that it can fully function in the more extreme temperatures and pressures it will face when deployed to the deepest of the hydrothermal vents.

Future work in the HVB project will now be in the bio-assay of the collected samples from the Arnarnesstrýturnar in the Eyjafjördur fjord in Iceland. The first analyses of the samples, the pH readings, indicate that the samples are pristine. Further analyses of the samples are underway to prove this and to see if any interesting microbes were sampled.

In the mean-time the HVB is getting ready for another test in the coming fall. It will then be attached to a Japanese ROV, called Hyper Dolphin, and descended to a depth of 2000-3000 meters for sampling the vent water of an over 200 °C deep-sea hydrothermal vent. The HVB system, including the nozzle, was proven at SIO to be fully functional at the pressures experienced at these depths. This deployment will test if the HVB system will operate at the higher temperature, as well as test the procedures for deploying the HVB with an ROV to a deep-sea vent. For this test the HVB will also be equipped with the additional two filter lines, making it a more complete system.

The HVB will hopefully help to shed some more light on the life at the hydrothermal vents on the bottom of the oceans and on how life on this planet could have been created in the first place. If microorganisms are found in the superheated vent water plumes from the deep-sea hydrothermal vents, a new record for extreme conditions where life can exist would be set. This would also give an indication that life might exist under similar conditions beyond Earth.

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